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Using 21st Century Science to Improve Risk-Related Evaluations

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Using 21st Century Science to Improve Risk-Related Evaluations

Committee on Incorporating 21st Century Science into Risk-Based Evaluations

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

A Report of

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Abbreviations

ACToR	Aggregated Computational Toxicology Resource
ADME	absorption, distribution, metabolism, and excretion
AHR	aryl-hydrocarbon receptor
AOP	adverse outcome pathway
B[a]P	benzo[a]pyrene
BPA	bisphenol A
Cas9	CRISPR associated protein 9
CC	collaborative cross
CCS	collisional cross-section
CDC	Center for Disease Control and Prevention
ChEMBL	Chemical European Molecular Biology Laboratory
CPT	Continuous Performance Test
CRISPR	clustered regularly interspaced short palindromic repeats
DNT	developmental neurotoxicity
DO	diversity outbred
DSSTox	distributed structure-searchable toxicity
ECHA	European Chemicals Agency
ECVAM	European Centre for the Validation of Alternative Methods
EFSA	European Food Safety Authority
EPA	US Environmental Protection Agency
ES21	<i>Exposure Science in the 21st Century: A Vision and a Strategy</i>
ESCAPE	European Study of Cohorts for Air Pollution Effects
EURL	European Union Reference Laboratory for Alternatives to Animal Testing
EWAS	exposome-wide association study
ExpoCast	exposure forecasting
FBS	fetal bovine serum
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GC	gas chromatography
GPCR	G-protein coupled receptors
GWAS	genome-wide association studies
HELIX	Human Early-Life Exposome Project
HERCULES	Health and Exposome Research Center: Understanding Lifetime Exposures
HMD	Human Metabolome Database
HTS	high-throughput screening

Abbreviations

IARC	International Agency for Research on Cancer
ICCVAM	Interagency Coordinating Committee on Validation of Alternative Methods
IMS	ion-mobility spectrometry
IOM	Institute of Medicine
iPSCs	induced pluripotent stem cells
IRIS	Integrated Risk Information System
IVIVE	in vitro in vivo extrapolation
LC	liquid chromatography
LDL	low-density lipoprotein
LUR	land-use regression
MCMH	4-methylcyclohexanemethanol
MS/MS	tandem mass spectrometry
NASA	National Aeronautics and Space Administration
NCATS	National Center for Advancing Translational Sciences
NHANES	National Health and Nutrition Examination Survey
NHGRI	National Human Genome Research Institute
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIH	National Institutes of Health
NIEHS	National Institute of Environmental Health Sciences
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRC	National Research Council
NTP	National Toxicology Program
OED	oral equivalent dose
OECD	Organisation for Economic Co-operation and Development
PAH	polycyclic aromatic hydrocarbon
PD	pharmacodynamics
PBPK	physiologically based pharmacokinetics
PhenX Toolkit	Phenotypes and Exposures ToolKit
PM	particulate matter
PPAR γ	peroxisome proliferator-activated receptor gamma
PPRTV	provisional peer reviewed toxicity value
PXR	pregnane X receptor
QSAR	quantitative structure–activity relationship
QSPR	quantitative structure–property relationship
RCPM	raven colored progressive matrices
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
RIX	recombinant inbred intercresses
rTK	reverse toxicokinetics
RXR	retinoid X receptor
SAP	Science Advisory Panel

Abbreviations

SAR	structure–activity relationship
SEEM	systematic empirical evaluation of models
SES	socioeconomic status
SEURAT	Safety Evaluation Ultimately Replacing Animal Testing
SHEDS-HT	Stochastic Human Exposure and Dose Simulation Model for High-Throughput
SHEDS-MM	Stochastic Human Exposure and Dose Simulation Model for Multimedia, Multipathway
STROBE	strengthening the reporting of observational studies in epidemiology
TCDD	tetrachlorodibenzo- <i>p</i> -Dioxin
TCE	trichloroethylene
Tox21	<i>Toxicity Testing in the 21st Century: A Vision and a Strategy Report</i>
ToxCast	Toxicity Forecaster
TTC	threshold of toxicological concern
WHO	World Health Organization
WPPSI	Wechsler Preschool and Primary Scale of Intelligence

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Using 21st Century Science to Improve Risk-Related Evaluations

Summary

At the start of the 21st century, several federal agencies and organizations began to recognize the potential of improving chemical risk assessment by using the scientific and technological advances in biology and other related fields that were allowing the biological basis of disease to be better understood. Substantial increases in computational power and advances in analytical and integrative methods made incorporating the emerging evidence into risk assessment a possibility. Strategies were developed to use the advances to improve assessment of the effects of chemicals or other stressors that could potentially affect human health. Building on those efforts, the National Research Council (NRC) report *Toxicity Testing in the 21st Century: A Vision and a Strategy*¹ envisioned a future in which toxicology relied primarily on high-throughput in vitro assays and computational models based on human biology to evaluate potential adverse effects of chemical exposures. Similarly, the NRC report *Exposure Science in the 21st Century: A Vision and a Strategy*² articulated a long-term vision for exposure science motivated by the advances in analytical methods, sensor systems, molecular technologies, informatics, and computational modeling. That vision was to inspire a transformational change in the breadth and depth of exposure assessment that would improve integration with and responsiveness to toxicology and epidemiology.

Since release of those two reports, government collaborations have been formed, large-scale US and international programs have been initiated, and data are being generated from government, industry, and academic laboratories at an overwhelming pace. It is anticipated that the data being generated will inform risk assessment and support decision-making to improve public health and the environment. In the meantime, questions have arisen as to whether or how the data now being generated can be used to improve risk-based decision-making. Because several federal agencies recognize the potential value of such data in helping them to address their many challenging tasks, the US Environmental Protection Agency (EPA), US Food and Drug Administration (FDA), National Institute of Environmental Health Sciences (NIEHS), and National Center for Advancing Translational Sciences (NCATS) asked the National Academies of Sciences, Engineering, and Medicine to recommend the best ways to incorporate the emerging science into risk-based evaluations.³ As a result of the request, the National Academies convened the Committee on Incorporating 21st Century Science into Risk-Based Evaluations, which prepared this report.

SCIENTIFIC ADVANCES

To approach its task, the committee assessed scientific and technological advances in exposure science and toxicology that could be integrated into and used to improve any of the four elements of risk assessment—hazard identification, dose–response assessment, exposure assessment, and risk characterization. Although the National Academies has not been asked to produce a report on epidemiology comparable with its Tox21 and ES21 reports, epidemiological research is also undergoing a transformation. Because it plays a critical role in risk assessment by providing human evidence on adverse effects of chemical and other exposures, the committee assessed advances in epidemiology as part of its charge. The committee highlights here some of the advances, challenges, and needs in each field in the context of risk assessment. The committee’s report provides specific recommendations to address the challenges. Over-

¹Referred to hereafter as the Tox21 report.

²Referred to hereafter as the ES21 report.

³The verbatim statement of task is provided in Chapter 1 of the committee’s report.

all, a common theme is the need for a multidisciplinary approach. Exposure scientists, toxicologists, epidemiologists, and scientists in other disciplines need to collaborate closely to ensure that the full potential of 21st century science is realized to help to solve the complex environmental and public-health problems that society faces.

Exposure Science

A primary objective for improving exposure science is to build confidence in the exposure estimates used to support risk-based decision-making by enhancing quality, expanding coverage, and reducing uncertainty. The many scientific and technological advances that are transforming exposure science should help to meet that objective. Some of the endeavors that the committee considered promising for advancing that objective and in which progress has been made since the ES21 report are highlighted below.

- *Remote sensing, personal sensors, and other sampling techniques.* Remote sensing enhances the capacity to assess human and ecological exposures by helping to fill gaps in time and place left by traditional ground-based monitoring systems. Advances in passive sampling techniques and personal sensors offer unparalleled opportunities to characterize individual exposures, particularly in vulnerable populations. If remote sensing and personal sensors can be combined with global positioning systems, exposure and human-activity data can be linked to provide a more complete understanding of human exposures.

- *Computational exposure tools.* Because exposure-measurement data on many agents are not available, recent advances in computational tools for exposure science are expected to play a crucial role in most aspects of exposure estimation for risk assessments, not just high-throughput applications. However, improving the scope and quality of data that are needed to develop parameters for these tools is critically important because without such data the tools have greater uncertainty and less applicability. Comparisons of calculated and measured exposures are required to characterize uncertainties in the computational tools and their input parameters.

- *Targeted and nontargeted analyses.* Advances in two complementary approaches in analytical chemistry are improving the accuracy and breadth of human and ecological exposure characterizations and are expanding opportunities to investigate exposure–disease relationships. First, targeted analyses focus on identifying selected chemicals for which standards and methods are available. Improved analytical methods and expanded chemical-identification libraries are increasing opportunities for such analyses. Second, nontargeted analyses offer the ability to survey more broadly the presence of all chemicals in the environment and in biofluids regardless of whether standards and methods are available. Nontargeted analyses reveal the presence of numerous substances whose identities can be determined after an initial analysis by using cheminformatic approaches or advanced or novel analytical techniques.

- *-Omics technologies.* -Omics technologies can measure chemical or biological exposures directly or identify biomarkers of exposure or response that allow one to infer exposure on the basis of a mechanistic understanding of biological responses. These emerging technologies and data streams will complement other analyses, such as targeted and nontargeted analyses, and lead to a more comprehensive understanding of the exposure-to-outcome continuum. Identifying biomarkers of exposure to individual chemicals or chemical classes within the complex exposures of human populations remains a considerable challenge for these tools.

- *Exposure matrices for life-span research.* Responding to the need to improve the characterization of fetal exposures to chemicals, researchers have turned to new biological matrices, such as teeth, hair, nails, placental tissue, and meconium. The growth properties (the sequential deposition or addition of tissue with accumulation of chemicals) and availability of the biospecimens offer the opportunity to extract a record of exposure. The question that needs to be addressed now is how concentrations in these matrices are related to and can be integrated with measures of exposure that have been traditionally used to assess chemical toxicity or risk.

- *Physiologically based pharmacokinetic (PBPK) models.* PBPK models are being applied more regularly to support aggregate (multiroute) exposure assessment, to reconstruct exposure from biomoni-

toring data, to translate exposures between experimental systems, and to understand the relationship between biochemical and physiological variability and variability in population response. An important focus has been on the development of PBPK models for translating exposures between test systems and human-exposure scenarios, development that has been driven by the rapidly expanding use of high-throughput in vitro assays to characterize the bioactivity of chemicals and other materials. That research will remain critical as regulatory agencies, industry, and other organizations increase their dependence on in vitro systems.

The emerging technologies and data streams offer great promise for advancing exposure science and improving and refining exposure measurements and assessment. However, various challenges will need to be addressed. A few are highlighted here.

- *Expanding and coordinating exposure-science infrastructure.* A broad spectrum of disciplines and institutions are participating in advancing exposure methods, measurements, and models. Given the number and diversity of participants in exposure science, the information is mostly fragmented, incompletely organized, and in some cases not readily available or accessible. Thus, an infrastructure is needed to improve the organization and coordination of the existing and evolving components for exposure science and ultimately to improve exposure assessment. Infrastructure development should include creating or expanding databases that contain information on chemical quantities in and chemical release rates from products and materials, on chemical properties and on processes, and analytical features that can be used in chemical identification.

- *Aligning environmental and test-system exposures.* Aligning information on environmental exposures with information obtained from experimental systems is a critical aspect of risk-based evaluation. Concentrations in test-system components need to be quantified by measurement, which is preferred, or by reliable estimation methods. Knowledge of physical processes, such as binding to plastic and volatilization, and of biological processes, such as metabolism, needs to be improved.

- *Integrating exposure information.* Integration and appropriate application of exposure data on environmental media, biomonitoring samples, conventional samples, and emerging matrices constitute a scientific, engineering, and big-data challenge. The committee emphasizes that integration of measured and modeled data is a key step in developing coherent exposure narratives, in evaluating data concordance, and ultimately in determining confidence in an exposure assessment. New multidisciplinary projects are needed to integrate exposure data and to gain experience that can be used to guide data collection and integration of conventional and emerging data streams.

Toxicology

The decade since publication of the Tox21 report has seen continued advances in an array of technologies that can be used to understand human biology and disease at the molecular level. Technologies are now available to profile the transcriptome, epigenome, proteome, and metabolome. There are large banks of immortalized cells collected from various populations to use for toxicological research; large compilations of publicly available biological data that can be mined to develop hypotheses about relationships between chemicals, genes, and diseases; and genetically diverse mouse strains and alternative species that can be used for toxicological research. Highlighted below are some assays, models, and approaches for predicting biological responses that have seen rapid advances over the last decade; they are arranged by increasing level of biological organization.

- *Probing interactions with biological molecules.* Chemical interactions with specific receptors, enzymes, or other discrete proteins and nucleic acids have long been known to have adverse effects on biological systems, and development of in vitro assays that probe chemical interactions with cellular components has been rapid, driven partly by the need to reduce high attrition rates in drug development. The assays can provide reliable and valid results with high agreement among laboratories and can be applied

in low-, medium-, and high-throughput formats. Computational models have been developed to predict activity of chemical interactions with protein targets, and research to improve the prediction of protein–chemical interactions continues.

- *Detecting cellular response.* Cell cultures can be used to evaluate a number of cellular processes and responses, including receptor binding, gene activation, cell proliferation, mitochondrial dysfunction, morphological changes, cellular stress, genotoxicity, and cytotoxicity. Simultaneous measurements of multiple toxic responses are also possible with high-content imaging and other novel techniques. Furthermore, cell cultures can be scaled to a high-throughput format and can be derived from genetically different populations so that aspects of variability in response to chemical exposure that depend on genetic differences can be studied. In addition to cell-based assays, numerous mathematical models and systems-biology tools have been advanced to describe various aspects of cell function and response.

- *Investigating effects at higher levels of biological organization.* The last decade has seen advances in engineered three-dimensional (3-D) models of tissues. Organotypic or organ-on-a-chip models are types of 3-D models in which two or more cell types are combined in an arrangement intended to mimic an in vivo tissue and, therefore, recapitulate at least some of the physiological responses that the tissue or organ exhibits in vivo. NCATS, for example, has a number of efforts in this field. Although the models are promising, they are not yet ready for inclusion in risk assessment. In addition to cell cultures, computational systems-biology models have been developed to simulate tissue-level response. EPA, for example, has developed virtual-tissue models for the embryo and liver. Virtual-tissue models can potentially help in conceptualizing and integrating current knowledge about the factors that affect key pathways and the degree to which pathways must be perturbed to activate early and intermediate responses in human tissues and, when more fully developed, in supporting risk assessments.

- *Predicting organism and population response.* Animal studies remain an important tool in risk assessment, but scientific advances are providing opportunities to enhance the utility of whole-animal testing. Gene-editing technologies, for example, have led to the creation of transgenic rodents that can be used to investigate specific questions, such as those related to susceptibility or gene–environment interactions. Genetically diverse rodent strains have provided another approach for addressing questions related to interindividual sensitivity to toxicants. Combining transgenic or genetically diverse rodent strains with -omics and other emerging technologies can increase the information gained from whole-animal testing alone. Those targeted studies can help to address knowledge gaps in risk assessment and can link in vitro observations to molecular, cellular, or physiological effects in the whole animal. In addition to the mammalian species, scientific advances have made some alternative species—such as the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the zebrafish *Danio rerio*—useful animal models for hazard identification and investigation of biological mechanisms.

The assays, models, and tools noted above hold great promise in the evolution of toxicology, but there are important technical and research challenges, a few of which are highlighted below.

- *Accounting for metabolic capacity in assays.* Current in vitro assays generally have little or no metabolic capability, and this aspect potentially constrains their usefulness in evaluating chemical exposures that are representative of human exposures that could lead to toxicity. Research to address the metabolic-capacity issues needs to have high priority, and formalized approaches need to be developed to characterize the metabolic competence of assays, to determine for which assays it is not an essential consideration, and to account for the toxicity of metabolites appropriately.

- *Understanding and addressing other limitations of cell systems.* Cell cultures can be extremely sensitive to environmental conditions, responses can depend on the cell type used, and current assays can evaluate only chemicals that have particular properties. Research is needed to determine the breadth of cell types required to capture toxicity adequately; cell batches need to be characterized sufficiently before, during, and after experimentation; and practical guidance will need to be developed for cell systems regarding their range of applicability and for describing the uncertainty of test results.

- *Addressing biological coverage.* Developing a comprehensive battery of in vitro assays that covers the important biological responses to the chemical exposures that contribute to adverse health effects is a considerable challenge. In addition, most assays used in the federal government high-throughput testing programs were developed by the pharmaceutical industry and were not designed to cover the full array of biological response. As emphasized in the Tox21 report, research is needed to determine the extent of relevant mechanisms that lead to adverse responses in humans and to determine which experimental models are needed to cover these mechanisms adequately. Using -omics technologies and targeted testing approaches with transgenic and genetically diverse rodent species and alternative species will address knowledge gaps more comprehensively.

When one considers the progress in implementing the Tox21 vision and the current challenges, it is important to remember that many assays, models, and tools were not developed with risk-assessment applications as a primary objective. Thus, understanding of how best to apply them and interpret the data is evolving. The usefulness or applicability of various in vitro assays will need to be determined by continued data generation and critical analysis, and some assays that are highly effective for some purposes, such as pharmaceutical development, might not be as useful for risk assessment of commodity chemicals or environmental pollutants. It will most likely be necessary to adapt current assays or develop new assays specifically intended for risk-assessment purposes.

Epidemiology

The scientific advances that have propelled exposure science and toxicology onto new paths have also substantially influenced the direction of epidemiological studies and research. The factors reshaping epidemiology in the 21st century include expansion of the interdisciplinary nature of the field; the increasing complexity of scientific inquiry; emergence of new data sources and technologies for data generation, such as new medical and environmental data sources and -omics technologies; advances in exposure characterization; and increasing demands to integrate new knowledge from basic, clinical, and population sciences. There is also a movement to register past and present datasets so that on particular issues datasets can be identified and combined.

One of the most important developments has been the emergence of the -omics technologies and their incorporation into epidemiological research. -Omics technologies have substantially transformed epidemiological research and advanced the paradigm of molecular epidemiology, which focuses on underlying biology (pathogenesis) rather than on empirical observations alone. The utility of -omics technologies in epidemiological research is already clear and well exemplified by the many studies that have incorporated genomics. For example, the genetic basis of disease has been explored in genome-wide association studies in which the genomic markers in people who have and do not have a disease or condition of interest are compared. The -omics technologies that have been applied in epidemiological research, however, have now expanded beyond genomics to include epigenomics, proteomics, transcriptomics, and metabolomics. New studies are being designed with the intent of prospectively storing samples that can be used for existing and future -omics technologies. Thus, obtaining data from human population studies that are parallel to data obtained from in vitro and in vivo assays or studies is already possible and potentially can help in harmonizing comparisons of exposure and dose. Furthermore, -omics technologies have the potential for providing a suite of new biomarkers for hazard identification and risk assessment.

Like exposure science and toxicology, epidemiology faces challenges in incorporating 21st century science into its practice. -Omics assays can generate extremely large datasets that need to be managed and curated in ways that facilitate access and analysis. Databases that can accommodate the large datasets, support analyses for multiple purposes, and foster data-sharing need to be developed. Powerful and robust statistical techniques also are required to analyze all the data. And standard ways to describe the data are needed so that data can be harmonized among investigative groups and internationally.

The landscape of epidemiological research is changing rapidly as the focus shifts from fixed, specific cohorts, such as those in the Nurses' Health Study,⁴ to large cohorts enrolled from health-care organizations or other resources that incorporate biospecimen banks and use health-care records to characterize participants and to track outcomes. Such studies offer large samples but will need new approaches to estimate exposures that will work in this context. Thus, there will be a need for close collaboration with exposure scientists to ensure that exposure data are generated in the best and most comprehensive way possible. Furthermore, various biospecimens are being collected and stored with the underlying assumption that they will be useful in future studies; researchers involved in such future-looking collections need to seek input from the scientists who are developing new assays so that the biospecimens can be collected and stored in a way that maximizes the potential for their future use. All those concerns emphasize the need to expand the multidisciplinary teams involved in epidemiological research.

APPLICATIONS OF 21st CENTURY SCIENCE

The scientific and technological advances described above and in further detail in this report offer opportunities to improve the assessment or characterization of risk for the purpose of environmental and public-health decision-making. The committee highlights below several activities—priority-setting, chemical assessment, site-specific assessment, and assessments of new chemistries—that could benefit from the incorporation of 21st century science. Case studies of practical applications are provided in Appendixes B–D.

Priority-setting has been seen as a principal initial application for 21st century science. High-throughput screening programs have produced toxicity data on thousands of chemicals, and high-throughput methods have provided quantitative exposure estimates. Several methods have been proposed for priority-setting, including risk-based approaches that use a combination of the high-throughput exposure and hazard information to calculate margins of exposure (differences between toxicity and exposure metrics). For that approach, chemicals that have a small margin of exposure would be seen as having high priority for further testing and assessment.

Chemical assessment is another activity in which the committee sees great potential for application of 21st century science. Chemical assessments encompass a broad array of analyses. Some cover chemicals that have a substantial database for decision-making, and for these assessments scientific and technical advances can be used to reduce uncertainties around key issues and to address unanswered questions. Many assessments, however, cover chemicals on which there are few data to use in decision-making, and for these assessments the committee finds an especially promising application for 21st century science. One approach for evaluating data-poor chemicals is to use toxicity data on well-tested chemicals (analogues) that are similar to the chemicals of interest in their structure, metabolism, or biological activity in a process known as read-across (see Figure S-1). The assumption is that a chemical of interest and its analogues are metabolized to common or biologically similar metabolites or that they are sufficiently similar in structure to have the same or similar biological activity. The method is facilitated by having a comprehensive database of toxicity data that is searchable by curated and annotated chemical structures and by using a consistent decision process for selecting suitable analogues. The approach illustrated in Figure S-1 can be combined with high-throughput *in vitro* assays, such as gene-expression analysis, or possibly with a targeted *in vivo* study to allow better selection of the analogues to ensure that the biological activities of a chemical of interest and its analogues are comparable. The committee notes that computational exposure assessment, which includes predictive fate and transport modeling, is an important complement to the approach described and can provide information on exposure potential, environmental persistence, and likelihood of bioaccumulation.

⁴The Nurses' Health Study is a prospective study that has followed a large cohort of women over many decades to identify risk factors for major chronic diseases.

Site-specific assessment represents another application for which 21st century science can play an important role. Understanding the risks associated with a chemical spill or the extent to which a hazardous-waste site needs to be remediated depends on understanding exposures to various chemicals and their toxicity. The assessment problem contains three elements—identifying and quantifying chemicals present at the site, characterizing their toxicity, and characterizing the toxicity of chemical mixtures—and the advances described in this report can address each element. First, targeted analytical-chemistry approaches can identify and quantify chemicals for which standards are available, and untargeted analyses can help to assign provisional identities to previously unidentified chemicals. Second, analogue-based methods coupled with high-throughput or high-content screening methods have the potential to characterize the toxicity of data-poor chemicals. Third, high-throughput screening methods can provide information on mechanisms that can be useful in determining whether mixture components might act via a common mechanism, affect the same organ, or cause the same outcome and thus should be considered as posing a cumulative risk. High-throughput methods can also be used to assess the toxicity of mixtures that are present at specific sites empirically rather than assessing individual chemicals.

Assessment of new chemistries is similar to the chemical assessment described above except that it typically involves new molecules on which there are no toxicity data and that might not have close analogues. Here, modern *in vitro* toxicology methods could have great utility by providing guidance on which molecular features are associated with greater or less toxicity and by identifying chemicals that do not affect biological pathways that are known to be relevant for toxicity. Modern exposure-science methods might also help to identify chemicals that have the highest potential for widespread environmental or human exposure and for bioaccumulation.

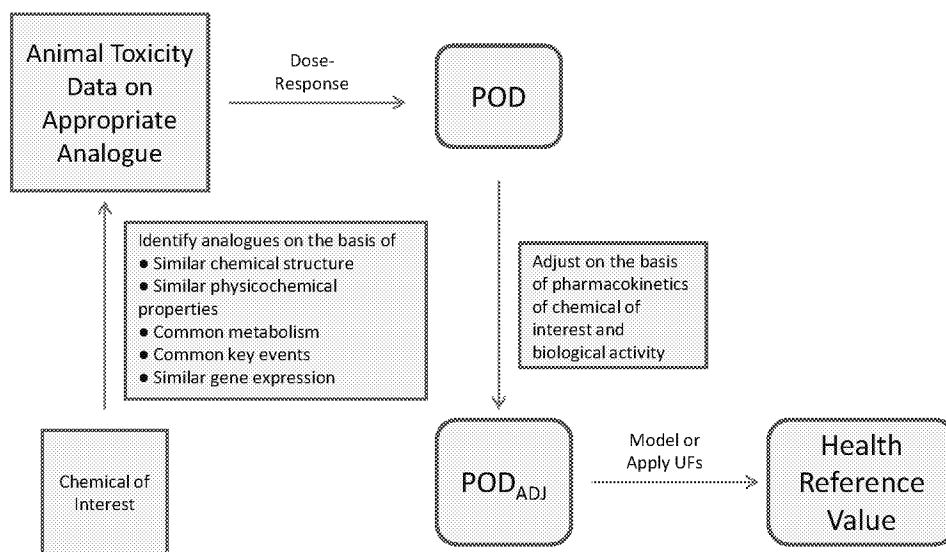


FIGURE S-1 Approach to deriving health reference values when data on similar chemicals are available. Similarity can be based on such characteristics as chemical structure, physicochemical properties, metabolism, key events in biological pathways, or gene expression; similarity of several characteristics increases confidence in the analogy. The point of departure (POD) of the appropriate analogue would be adjusted on the basis of pharmacokinetic differences between the chemical of interest and the analogue and other important biological factors, such as receptor activation; relevant uncertainty factors would then be applied or models would be used to derive the health reference value. Accounting for uncertainty could include a determination of the degree of confidence in the read-across, including the number of analogues identified, the degree of similarity of the analogues to the chemical of interest, and the extent of the dataset on the analogues.

VALIDATION

Before new assays, models, or test systems can be used in regulatory-decision contexts, it is expected and for some purposes legally required that their relevance, reliability, and fitness for purpose be established and documented. That activity has evolved into elaborate processes that are commonly referred to as validation of alternative methods. One critical issue is that current processes for validation cannot match the pace of development of new assays, models, and test systems, and many have argued that validation processes need to evolve. Important elements of the validation process that need to be addressed include finding appropriate comparators for enabling fit-for-purpose validation of new test methods, clearly defining assay utility and how assay data should be interpreted, establishing performance standards for assays and clear reporting standards for testing methods, and determining how to validate batteries of assays that might be used to replace toxicity tests. The committee discusses those challenges further and offers some recommendations in Chapter 6.

A NEW DIRECTION FOR RISK ASSESSMENT AND THE CHALLENGES IT POSES

The advances in exposure science, toxicology, and epidemiology described in this report support a new direction for risk assessment, one based on biological pathways and processes rather than on observation of apical responses and one incorporating the more comprehensive exposure information emerging from new tools and approaches in exposure science. The exposure aspect of the new direction focuses on estimating or predicting internal and external exposures to multiple chemicals and stressors, characterizing human variability in those exposures, providing exposure data that can inform toxicity testing, and translating exposures between test systems and humans. The toxicology and epidemiology elements of the new direction focus on the multifactorial and nonspecific nature of disease causation; that is, stressors from multiple sources can contribute to a single disease, and a single stressor can lead to multiple adverse outcomes. The question shifts from whether A causes B to whether A increases the risk of B. The committee found that the sufficient-component-cause model, which is illustrated in Figure S-2, is a useful tool for conceptualizing the new direction. The same outcome can result from more than one causal complex or mechanism; each mechanism generally involves joint action of multiple components.

Most diseases that are the focus of risk assessment have a multifactorial etiology; some disease components arise from endogenous processes, and some result from the human experience, such as background health conditions, co-occurring chemical exposures, food and nutrition, and psychosocial stressors. Those additional components might be independent of the environmental stressor under study but nonetheless influence and contribute to the overall risk and incidence of disease. As shown in the case

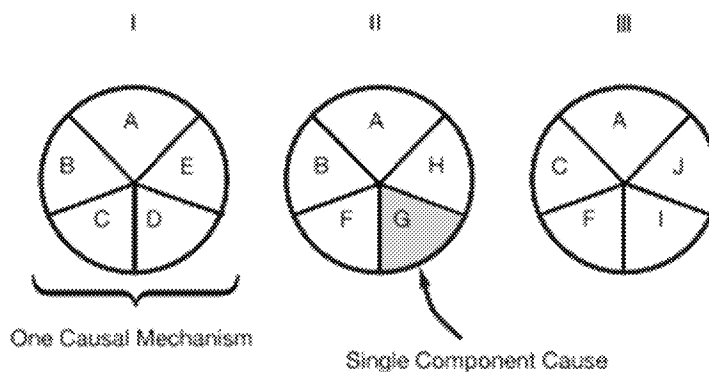


FIGURE S-2 Multifactorial nature of disease illustrated by using the sufficient-component-cause model in which various overall mechanisms (I, II, and III) for a disease are represented as causal pies of various components (A–J). The committee considers pathways to be components of the mechanism.

studies in this report, one does not need to know all the pathways or components involved in a particular disease to begin to apply the new tools to risk assessment. The 21st century tools provide the mechanistic and exposure data to support dose–response characterizations and human-variability derivations described in the NRC report *Science and Decisions: Advancing Risk Assessment*. They also support the understanding of relationships between disease and components and can be used to probe specific chemicals for their potential to perturb pathways or activate mechanisms and increase risk.

The 21st century science with its diverse, complex, and very large datasets, however, poses challenges related to analysis, interpretation, and integration of data and evidence for risk assessment. In fact, the technology has evolved far faster than the approaches for those activities. The committee found that Bradford-Hill causal guidelines could be extended to help to answer such questions as whether specific pathways, components, or mechanisms contribute to a disease or outcome and whether a particular agent is linked to pathway perturbation or mechanism activation. Although the committee considered various methods for data integration, it concluded that guided expert judgment should be used in the near term for integrating diverse data streams for drawing causal conclusions. In the future, pathway-modeling approaches that incorporate uncertainties and integrate multiple data streams might become an adjunct to or perhaps a replacement for guided expert judgment, but research will be needed to advance those approaches. The committee emphasizes that insufficient attention has been given to analysis, interpretation, and integration of various data streams from exposure science, toxicology, and epidemiology. It proposes a research agenda that includes developing case studies that reflect various scenarios of decision-making and data availability; testing case studies with multidisciplinary panels; cataloguing evidence evaluations and decisions that have been made on various agents so that expert judgments can be tracked and evaluated, and expert processes calibrated; and determining how statistically based tools for combining and integrating evidence, such as Bayesian approaches, can be used for incorporating 21st century science into all elements of risk assessment.

CONCLUDING REMARKS

As highlighted here and detailed in the committee's report, many scientific and technical advances have followed publication of the Tox21 and ES21 reports. The committee concludes that the data that are being generated today can be used to address many of the risk-related tasks that the agencies face, and it provides several case studies in its report to illustrate the potential applications. Although the challenges to achieving the visions of the earlier reports often seem daunting, 21st century science holds great promise for advancing risk assessment and ultimately for improving public health and the environment. The committee emphasizes, however, that communicating the strengths and limitations of the approaches in a transparent and understandable way will be necessary if the results are to be applied appropriately and will be critical for the ultimate acceptance of the approaches.

Introduction

Over the last decade, several large-scale US and international programs have been initiated to incorporate advances in molecular and cellular biology, -omics technologies, analytical methods, bioinformatics, and computational tools and methods into the field of toxicology. The overarching goal of the various programs is to move toxicology from a practice that uses whole-animal testing to one that uses primarily modern in vitro assays and computational approaches to predict toxicity on the basis of an understanding of the biological processes that ultimately lead from the initial chemical exposure to adverse effects. Similar efforts are being pursued in the field of exposure science with the goals of obtaining more accurate and complete exposure data on individuals and populations for thousands of chemicals over the lifespan; predicting exposures from use data and chemical-property information; and translating exposures between test systems and humans. It is hoped that the advances in toxicology and exposure science and better integration of the fields will improve risk assessment and thus better support decision-making to improve public and environmental health. With various efforts under way, diverse data are being generated, and their utility for risk assessment investigated. Although the programs and the data being generated are still evolving and will undoubtedly continue to do so, some data could be used now to help to fill gaps and assess chemical risk better. Several federal agencies recognize the potential value of such data in helping them to address their many challenging tasks. Accordingly, the US Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), the National Institute of Environmental Health Sciences (NIEHS), and the National Center for Advancing Translational Sciences (NCATS) asked the National Academies of Sciences, Engineering, and Medicine to consider the integration of modern and emerging scientific approaches and data into risk-based evaluations and to recommend the best ways to do so. As a result of the request, the National Academies convened the Committee on Incorporating 21st Century Science into Risk-Based Evaluations, which prepared this report.

TOXICOLOGY IN THE 21st CENTURY

In the early 2000s, several agencies and organizations began to recognize the potential of various scientific advances in biology and related fields and the possibilities provided by increases in computational power to characterize risks of environmental exposures. Roadmaps were developed to incorporate such advances into their strategic plans for assessing chemicals and other agents (EPA 2003; NTP 2004). In 2007, the National Research Council (NRC) released the report *Toxicity Testing in the 21st Century: A Vision and a Strategy*,¹ which envisioned transforming toxicity testing from a system that relies on animal assays to one that relies primarily on high-throughput in vitro assays and computational methods based on human biology. The primary goals behind the vision were “(1) to provide broad coverage of chemicals, chemical mixtures, outcomes, and life stages, (2) to reduce the cost and time of testing, (3) to use fewer animals and cause minimal suffering in the animals used, and (4) to develop a more robust scientific basis for assessing health effects of environmental agents” (NRC 2007). The committee that prepared the 2007 report emphasized that the transformation would require a focused effort over several decades for full implementation. On release of the report, the NIEHS National Toxicology Program, the EPA National Center for Computational Toxicology, and the Chemical Genomics Center² of the National Institutes of

¹Referred to hereafter as the Tox21 report.

²The Chemical Genomics Center is now part of NCATS.

Health formed a collaboration, known as Tox21, to advance the vision set forth in the 2007 report (Collins et al. 2008). FDA later joined the collaboration.

The goals of the Tox21 collaboration are to identify and characterize specific mechanisms or pathways that lead to adverse effects in humans, to design assays to measure pathway responses, to develop models that can predict toxicity using the assay data, and to set priorities among chemicals for more comprehensive toxicity testing (NCATS 2015a). It is planned that the data generated will ultimately help to inform EPA, FDA, and other agencies on the hazards posed by the chemicals or products that they regulate and will be used by industry to screen for potential toxicity in product development. A phased approach to the research is being taken. Phase I of Tox21 has been completed and involved testing of about 2,800 chemicals in about 50 assays, including ones to assess cytotoxicity, mitochondrial toxicity, cell signaling, DNA damage, immune response, drug metabolism, nuclear-receptor activation, and inhibition of various molecular targets (Tice et al. 2013; NCATS 2015b). Phase II involves testing of over 10,000 chemicals that occupy a diverse chemical and toxicological space and include “industrial chemicals, sunscreen additives, flame retardants, pesticides and selected metabolites, plasticizers, solvents, food additives, natural product components, drinking water disinfection by-products, preservatives, therapeutic agents, and chemical synthesis by-products” (Tice et al. 2013). Phase III will involve identification of physiologically relevant cells, measurement of gene expression in a large number of molecular pathways, and testing of chemical mixtures and extracts (NCATS 2015b).

In 2007, EPA initiated its Toxicity Forecaster (ToxCast) program, which seeks to develop high-throughput screening (HTS) assays for evaluating biological responses that are relevant to prediction of adverse effects of chemical exposures on humans (EPA 2013). A phased approach to research is also being taken in the ToxCast program. Phase I, which has been completed, involved testing of over 300 well-studied chemicals in several hundred HTS assays (Kavlock and Dix 2010). Phase II has also been completed; it involved testing of over 2,000 chemicals—including industrial and consumer products, food additives, and potentially safer chemical alternatives to existing chemicals—in HTS assays for evaluating various cell responses and over 300 signaling pathways (EPA 2013; Silva et al. 2015). ToxCast data are now being evaluated as a means of setting priorities among chemicals for testing in EPA’s Endocrine Disruptor Screening Program and in other programs that require setting priorities for testing.

In addition to US government-led efforts, international efforts are transforming toxicology from an observational to a predictive science. In the European Union, for example, the European Commission and Cosmetics Europe (a trade association for the cosmetics and personal-care industry) have co-funded the research initiative Safety Evaluation Ultimately Replacing Animal Testing (SEURAT 2015). The initiative was started to develop tools to comply with legislation that banned all animal testing for cosmetic ingredients and all marketing of animal-tested cosmetic ingredients and products; a complete ban went into effect in March 2013. Its vision was to eliminate traditional animal testing by adopting a “toxicological mode-of-action framework to describe how any substance may adversely affect human health, and use this knowledge to develop complementary theoretical, computational and experimental (in vitro) models that predict quantitative points of departure needed for safety assessment” (Berggren 2015). The research initiative was a 5-year program (2011-2015) that involved development of in vitro assays that use human pluripotent stem cells, development of a hepatic microfluidic bioreactor, identification and investigation of human biomarkers of chronic toxicity in cellular models, and development of computational tools for predicting chronic toxicity.

Private industry and other organizations are also working to transform the ways in which chemicals are assessed. For example, the pharmaceutical industry has been developing and using in vitro and computational tools as early screens for drug safety for many years (Greene and Song 2011; Bowes et al. 2012). Organizations have developed case studies related to the use of new in vitro assays and computational systems-biology tools for assessment of chemical risk (Daston et al. 2015; Gocht et al. 2015). Cheminformatics research has resulted in the development of rational systems for informing qualitative structure–activity relationship assessments (Wu et al. 2010) and in the development of automated decision trees for identifying toxicity end points, such as developmental and reproductive toxicity (Wu et al. 2013).

Academic institutions are generating a substantial amount of data that could help to inform chemical risk assessment. Academic laboratories tend to focus on end points that are not typically covered in guideline animal studies, such as mammary gland development (Fenton 2006; Soto et al. 2008; Osborne et al. 2015), synaptic morphology and other aspects of nervous system development (Patisaul and Polston 2008), and complex behaviors, including sociality, aggression, cognition, and behavioral hallmarks of psychiatric disorders, such as autism spectrum disorder and attention deficit disorder (Eubig et al. 2010; de Cock et al. 2012; Leon-Olea et al. 2014). Research on genetics, genomics, and epigenetics (including the role of noncoding RNAs) is also abundant and is providing insights on novel biological mechanisms and gene-by-environment interactions (Dolinoy et al. 2007; Rusyn et al. 2010; Tal and Tanguay 2012; Nebert et al. 2013; Yeo et al. 2013). Academic laboratories have been responsible for generating nearly all the data on transgenerational effects (Rissman and Adli 2014); have pioneered the use of nontraditional animal models, including transgenic and population-based models (Churchill et al. 2004; Rusyn et al. 2010; Sullivan et al. 2014); and have conducted most of the epidemiological studies of chemical risk. The enormous volume of data being generated throughout the basic- and clinical-research communities has prompted questions about how the data could best be used for various risk-related activities and decision-making.

EXPOSURE SCIENCE IN THE 21st CENTURY

Exposure science is undergoing a transformation similar to that affecting toxicology with the advances in molecular technologies, computational tools, bioinformatics, sensor systems, and analytical methods. In 2012, NRC released the report *Exposure Science in the 21st Century: A Vision and a Strategy*,³ which articulated a long-term vision for exposure science. The primary long-term goal of the vision was to broaden the reach of exposure science from a traditional focus on discrete exposures to an “integrated approach that considers exposures from source to dose, on multiple levels of integration (including time, space, and biological scale), to multiple stressors, and scaled from molecular systems to individuals, populations, and ecosystems” (NRC 2012). The report described scientific and technological progress that has the potential to transform exposure science, including geographic information technologies that can track sources, exposure concentrations, and receptors; monitoring technologies that can collect data on personal exposure of millions of people; highly sensitive analytical technologies that can identify and measure biomarkers that are indicative of internal exposures; and computational tools that can manage the large amounts of data generated. It also highlighted high-priority research, emphasized the need for inter-agency collaboration and resources, and elaborated the broad concept of the exposome, defined as “the record of all exposures both internal and external that people receive throughout their lifetime (Rappaport and Smith 2010).” Last, it recognized the interdependence of the fields of toxicology, risk assessment, and exposure science and foresaw the need to evolve the risk-assessment paradigm toward one in which exposure science plays a strong role, specifically, a paradigm that is “influenced by and responsive to human and environmental exposure data.” The report described four objectives of exposure science: to set priorities among chemicals for toxicity testing; to provide exposure information to guide toxicity testing; to provide quantitative pharmacokinetic data on absorption, distribution, metabolism, and excretion (ADME) derived from human-exposure studies; and to connect exposure data with biological activity data to identify exposure–response relationships.

In response to the recommendation to improve integration of exposure science throughout the federal government, the Exposure Science in the 21st Century (ES21) Federal Working Group has emerged (EPA 2016a). It consists of representatives of more than 20 federal organizations that have a common interest in exposure-science research and development. The purpose of the working group is to build on the framework recommended in the ES21 report, share information, integrate activities, reduce duplication of efforts among agencies, and promote federal collaboration in the development of exposure science. In addition to the activities of the working group, several research programs are involved in advancing

³Referred to hereafter as the ES21 report.

exposure science on paths that are consistent with the vision articulated in the ES21 report. EPA created the Exposure Forecasting (ExpoCast) program, which complements its ToxCast program (EPA 2016b). ExpoCast focuses on developing high-throughput methods for estimating exposure and so far has been used to make exposure predictions related to over 1,900 chemicals. EPA's goal is to combine the exposure estimates from ExpoCast with bioactivity data from ToxCast to predict human health and environmental risks.

NIEHS is also interested in advancing exposure science and has supported research to develop new sensor systems and to identify biomarkers of response to exposure (NIEHS 2015). It has created the Children's Health Exposure Analysis Resource (NIEHS 2016), an infrastructure designed to enable and expand incorporation of environmental exposures into studies of children's health; it includes a data repository, support for statistical analysis, and a network of laboratories to analyze biological samples. The NIEHS strategic plan emphasizes a commitment to supporting research to define and explore the exposome, and the agency has funded the HERCULES center at Emory University to conduct exposome-focused research (NIEHS 2012).

In addition to the efforts in the United States, there are international efforts, such as the Human Early-Life Exposome (HELIX) project and the EXPOsOMICS project. HELIX has the ambitious goal of characterizing early-life exposures and ultimately linking exposures with children's health outcomes (Vrijheid et al. 2014). The project is studying 32,000 mother-child pairs in six European countries. EXPOsOMICS focuses on the external and internal exposome associated with air pollution and water contamination (Vineis et al. 2013, in press). The project will perform personal-exposure monitoring of air pollutants for hundreds of subjects in Europe, and biological samples from thousands of subjects will be analyzed for internal exposure markers by using -omics technologies (CORDIS 2015).

Like the toxicology initiatives, the exposure programs are generating vast amounts of data, but how the data are best used to inform risk-related tasks and decision-making remains to be determined.

TERMINOLOGY

The recent advances in toxicology and exposure science have given rise to a new vocabulary and a plethora of new terms. Some researchers and practitioners distinguish between terms, but others use the same terms interchangeably and inconsistently. Consequently, there is some confusion as to the specific meanings of various terms. *Mode of action*, *mechanism of action*, and *adverse outcome pathway* are exemplary of the confusion. Each term denotes a progression from some exposure or molecular initiating event to an adverse outcome. *Mechanism of action* is often distinguished from *mode of action* by a greater level of biological detail in the understanding and description of the progression from exposure to outcome (EPA 2005; NRC 2007). *Mode of action* typically describes the progression of key events that result from a chemical exposure whereas *adverse outcome pathway* conceptually describes the sequential chain of causally linked events at various levels of biological organization starting from a molecular initiating event through to the observable adverse outcome (OECD 2013; Berggren et al. 2015). Although all three terms are used to describe the sequence of steps from an initiating event to an adverse outcome, subtle distinctions between the terms have been made. The subtleties are often lost in practice, and the terms are used interchangeably. In the present report, the committee uses primarily *mechanism* and defines the term generally to refer to a detailed description of the process by which an agent causes an effect. It uses *adverse outcome pathway* only in the context of frameworks that have been developed specifically with the phrase. Mechanism is further defined in the context of the new direction of risk assessment in Chapters 5 and 7.

Exposure and *dose* are two other terms that are often defined and used inconsistently. NRC (2012) defined exposure broadly as the contact between a stressor and a receptor at any level of biological organization (organism, organ, tissue, or cell). Given that broad definition, the distinction between *exposure* and *dose* becomes arbitrary, and *dose* becomes unnecessary. Exposure is then characterized by the identity of the stressor and the amount, location, and timing of the stressor that comes into contact with the receptor; timing encompasses both duration and the time at which the contact occurs. The committee uses

exposure primarily in the present report but acknowledges that it often uses dose in conventional phrases, such as dose–response relationship.

Many terms associated with -omics technologies have been coined in recent years. Box 1-1 provides definitions of various terms used throughout this report. Other terms that are specific to topics discussed in various chapters are defined in those chapters. The committee acknowledges that as the science progresses new terms will be needed, but it urges the scientific community to be judicious in inventing new terms. If needed, new terms should be defined clearly and used consistently.

The committee debated how to refer to all the assays, tools, and methods arising from the “21st century visions” for toxicology and exposure science; some are no longer “new,” and others are still in development. To simplify the text, the committee often refers to them as Tox21 or ES21 assays, tools, or methods. That notation is meant to be broad and includes all the assays, tools, and methods coming from government, academic, and private laboratories, not only those being developed as part of the Tox21 program previously described.

THE COMMITTEE AND ITS TASK

The committee that was convened as a result of the agencies’ request included experts in toxicology; physiologically based pharmacokinetic modeling; computational methods and bioinformatics; -omics, in vitro models, and alternative methods; epidemiology; exposure assessment; statistics; and risk assessment (see Appendix A for the committee’s biographical information). As noted, the committee was asked to consider and recommend the best uses of the various types of emerging data in risk-based evaluations. The committee’s verbatim statement of task is provided in Box 1-2.

BOX 1-1 Definitions of Various -Omics Terms

Adductomics: The comprehensive identification of chemicals that bind to DNA or selected proteins, such as albumin.

Epigenomics: The analysis of epigenetic changes in DNA, histones, and chromatin that regulate gene expression. Epigenetic changes are changes other than changes in DNA sequence that are involved in gene silencing.

Exposome: A term first coined by Wild (2005) to represent the totality of a person’s exposure from conception to death; exposome research involves the measurement of multiple exposure indicators by using -omics approaches.

Genomics: The analysis of the structure and function of genomes.

Metabolomics: The scientific study of small molecules (metabolites) that are created from chemicals that originate inside the body (endogenously) or outside the body (exogenously) (National Academies of Sciences, Engineering, and Medicine 2016). For purposes of the present report, metabolomics is assumed to include exogenous chemicals found in biological systems in their unmetabolized forms.

Proteomics: The analysis of the proteins produced by cells, tissues, or organisms. Analysis is conducted to understand the location, abundance, and post-translational modification of proteins in a biological sample.

Transcriptomics: Qualitative and quantitative analysis of the transcriptome, that is, the set of transcripts (mRNAs, noncoding RNAs, and miRNAs) that is present in a biological sample.

BOX 1-2 Statement of Task

An ad hoc committee under the auspices of the National Research Council (NRC) will provide recommendations on integrating new scientific approaches into risk-based evaluations. Specifically, the committee will first consider the scientific advances that have occurred following the publication of the NRC reports *Toxicity Testing in the 21st Century: A Vision and a Strategy* and *Exposure Science in the 21st Century: A Vision and a Strategy*. Given the various ongoing lines of investigation and new data streams that have emerged, the committee will then propose how best to integrate and use the emerging results in evaluating chemical risk and identify how traditional human-health risk assessment can incorporate the new science. It will consider whether a new paradigm is needed for data validation (or acceptance), how to integrate the divergent data streams, how uncertainty might need to be characterized (or how characterization of uncertainty might need to change), and how best to communicate the new approaches so that they are understandable to various stakeholders. It will focus its recommendations on pragmatic solutions and provide case studies that illustrate its recommendations. Finally, the committee will identify barriers or obstacles to advancing and integrating the various types of science, and ultimately transforming risk assessment.

THE COMMITTEE'S APPROACH TO ITS TASK

To address its task, the committee held seven meetings, which included three open sessions to hear primarily from various sponsor representatives. Given the potential breadth of its task, the committee devoted substantial time to interpretation of its charge. It used as a basis of its work the risk-assessment framework that was initially proposed in the 1983 report *Risk Assessment in the Federal Government: Managing the Process* (NRC 1983) and updated most recently in the 2009 report *Science and Decisions: Advancing Risk Assessment* (NRC 2009) (see Figure 1-1). The committee considered and describes scientific and technological advances in exposure science, toxicology, and epidemiology that could be integrated into and used to improve any of the four elements of risk assessment (hazard identification, dose-response assessment, exposure assessment, and risk characterization). The report, however, is not a catalog of all scientific and technological advances that have been made since publication of the 2007 and 2012 reports (NRC 2007, 2012), but rather a review of the ones most relevant to risk-based evaluations in EPA and FDA.

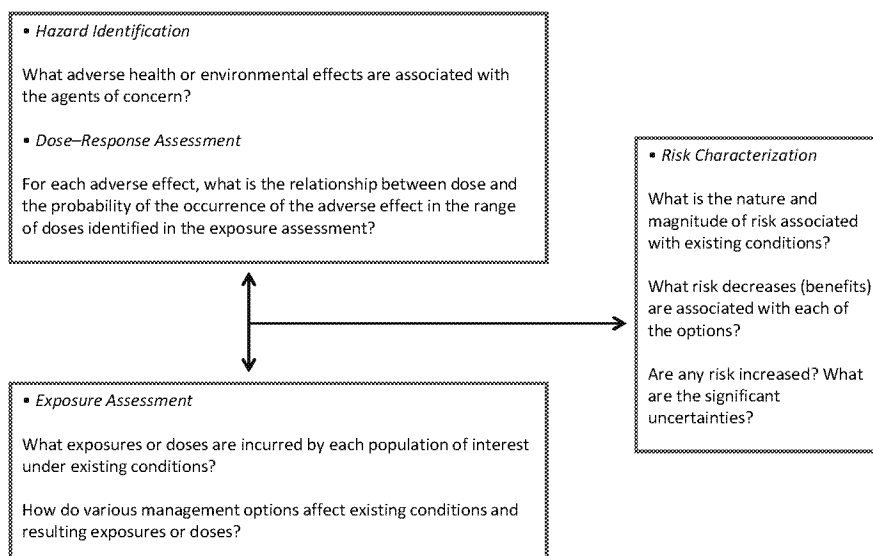


FIGURE 1-1 The risk-assessment process as defined by its four elements: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Source: Adapted from NRC 2009.

The committee identified various agency tasks and decision-making contexts (see Box 1-3)—which require different depths of information—and used the tasks and contexts to frame general and specific examples of applications (case studies) for integrating the new science into various components of risk assessment. The examples provide guidance for communicating to various stakeholders how the new science could be used. The committee then considered how data validation, data integration, and uncertainty analysis might need to be adapted to use the new science. The committee recognizes that there will be challenges in using new tools and concepts in fields that are already heavy with practice standards and set protocols.

BOX 1-3 Agency Tasks and Decision-Making Contexts

- 1) Priority-setting—Can be based on hazard, exposure, or risk.
- 2) Chemical assessment—Can include Integrated Risk Information System assessments, Provisional Peer Reviewed Toxicity Values, National Toxicology Program Office of Health Assessment and Translation hazard assessments, and assessments of various regulated substances, such as pesticides, drugs, and food additives.
- 3) Site-specific assessments—Can involve selection of geographic sites or chemicals at a site to evaluate and can involve assessment of data-poor chemicals or mixtures; can also involve assessment of previously unidentified chemicals in the environment.
- 4) Assessment of new chemistries—Can involve assessment of green chemistry, new-to-the-world technologies, and unexpected environmental degradation products of chemicals in commerce.

ORGANIZATION OF THIS REPORT

The committee's report is organized into seven chapters and five appendixes. Chapters 2, 3, and 4 describe new or emerging methods and tools in exposure science, toxicology, and epidemiology, respectively. Chapter 5 highlights the new direction of risk assessment and describes practical applications for 21st century science. Chapter 6 discusses issues surrounding model and assay validation and acceptance. Chapter 7 focuses on interpretation and integration of data and evidence. Appendix A provides biographical information on the committee members, and Appendixes B, C, and D provide case studies that demonstrate practical applications of the committee's recommendations for using new data streams in risk-based evaluations. Appendix E provides a case study in using Bayesian approaches with high-throughput data.

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Advances in Exposure Science

As described in Chapter 1, the National Research Council (NRC) report *Exposure Science in the 21st Century: A Vision and a Strategy* articulated a vision for exposure science that was intended to transform, expand, and invigorate the field (NRC 2012). Recent investments in exposome technologies and programs (CHEAR; NIEHS 2016), in new large-scale longitudinal exposure-epidemiology research programs (HELIX; Vrijheid et al. 2014 and EXPOsOMICS; Vineis et al. 2013), and in the rapidly expanding exposure-science programs headed by the National Exposure Research Laboratory and the National Center for Computational Toxicology of the US Environmental Protection Agency (EPA) are examples of the immediate impact of the ES21 report.¹ Several research fields have seen substantial advances since the ES21 report was published, and these advances create opportunities for providing guidance to EPA, the Food and Drug Administration, and others on how best to integrate emerging exposure-science data into risk assessments (Egheghy et al. 2016). Accordingly, this chapter describes the major advances in exposure science since the publication of the ES21 report and applications that would be most relevant and useful for risk-based decision-making. It also presents unaddressed opportunities related to decision-making based on exposure or risk and discusses major obstacles to various applications.

The interrelationship among the fields of exposure science, toxicology, and epidemiology is a central theme of this chapter. Figure 2-1 illustrates the series of events from introduction of a stressor into the environment and its movement through the environment via specific pathways to the receptor and the triggering of a biological response of potential regulatory concern. The figure provides a broad conceptual overview of the scope of exposure science and a general organizational framework as envisaged by the ES21 committee and the present committee. The figure also illustrates the points of integration with toxicology and epidemiology and the fundamental distinctions between fields. Although the continuum is depicted as a linear path, the committee recognizes that multiple interconnecting paths are typically involved in the source-to-outcome continuum. In cases where source identification or mitigation rather than toxicology or risk assessment is the goal, one moves from right to left from measured exposures to sources. Box 2-1 provides some definitions of the key terms used in this chapter related to exposure science.

Organizational frameworks for exposure science, such as the one in Figure 2-1, have been used to describe exposure pathways for contaminated sites and are implicit in all models of environmental or biological fate of chemicals (Wania and Mackay 1999; Koelmans et al. 2001; Schenker et al. 2009). The frameworks have been essential in guiding the acquisition of data, the organization of data, and the use of data in modeling to describe or predict exposure quantitatively. Although some frameworks, such as the Conceptual Site Model (Regens et al. 2002; Mayer et al. 2005), are largely qualitative and conceptual and apply to specific exposure settings or specifically to modeling exercises, others, such as the Aggregate Exposure Pathway framework (Teeguarden et al. 2016), attempt to expand on earlier successes by generalizing the approach to support data acquisition, data organization, conceptualization, and modeling in the broader exposure-science community. As the field of exposure science evolves as a result of advances in the tools and approaches described in this chapter, the use of the frameworks will enable the development of infrastructure to support exposure-data acquisition, collection, organization, and access and to improve the accuracy, completeness, efficiency, and transparency of exposure assessment and modeling.

¹The present committee refers to *Exposure Science in the 21st Century: A Vision and a Strategy* (NRC 2012) as the ES21 report and to its committee as the ES21 committee.

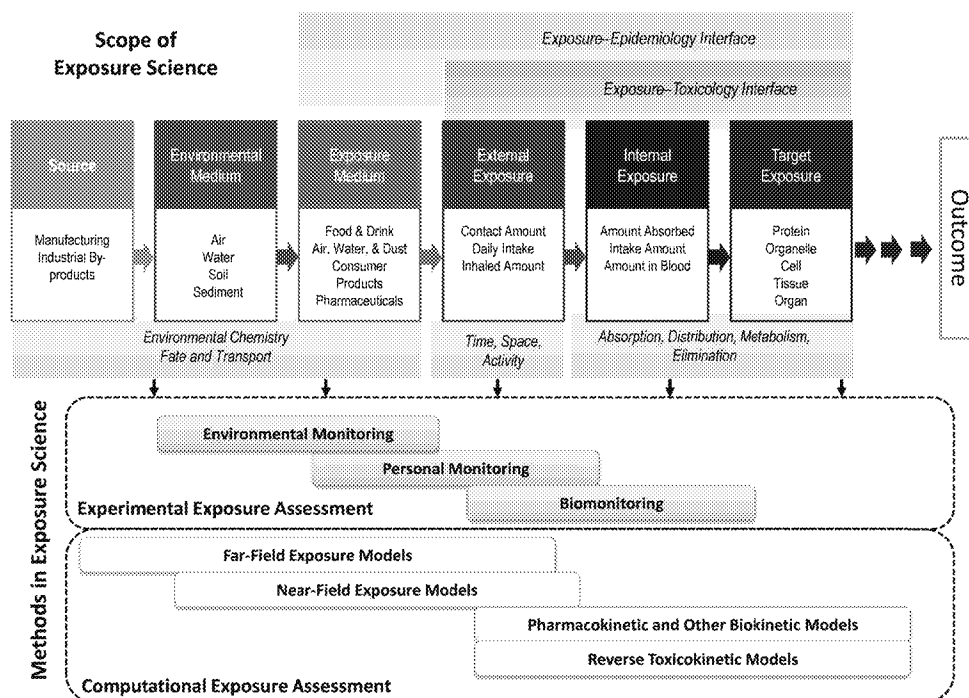


FIGURE 2-1 Conceptual overview of the scope of and common methods for exposure science. Toxicology and epidemiology have traditionally used both internal-exposure and external-exposure information. The biological interface between exposure and a receptor (such as a human, tissue, or cell) is the test-system or target-site exposure. The main benefit of applying target-site exposures is a reduction in confounding by pharmacokinetic and other factors and has led to increasing use of target-site exposure metrics in toxicology and epidemiology.

MAJOR ADVANCES IN EXPOSURE SCIENCE

The committee reviewed advances in the field of exposure science since the publication of the ES21 report with the goal of identifying major advances that have the potential for sustained effects on the important applications described later in this chapter and in the case studies described in Appendixes B–D. The advances are summarized in this section.

Remote Sensing and Geospatial Environmental Exposure Assessment

Several substantial advances in exposure science are the result of innovations in remote sensing, global positioning systems (GPS), and geographic information systems (GIS). Remote sensing is an important tool for enhancing the capacity to assess human and ecological exposures because it provides information on Earth's surface, water, and atmosphere that cannot be provided by traditional ground-based monitoring systems (Al-Hamdan et al. 2014). Since the ES21 report, remote-sensing data have been used to estimate concentrations of ambient criteria air pollutants (NO_2 , O_3 , and $\text{PM}_{2.5}$) on a global scale (Brauer et al. 2015; Geddes et al. 2016; van Donkelaar et al. 2015). Models have estimated the changes in global air pollution and have allowed complete global coverage of key pollutants on a relatively fine spatial scale. The application of remote-sensing technologies with ground-based monitoring will continue to improve human exposure assessment. Several recent key advances include the National Aeronautics and Space Administration (NASA) launch of six Earth-observing missions and the addition of three new instruments to the International Space Station (Seltenrich 2014). NASA and the National Oceanic and Atmospheric Administration provide free access to exposure-relevant data, such as NO_2 and $\text{PM}_{2.5}$ concentrations in the troposphere, and environmental data relevant to exposure assessment and interpretation of monitoring data (Seltenrich 2014).

BOX 2-1 Definitions of Selected Exposure Terms

Exposure science. “The collection and analysis of quantitative and qualitative information needed to understand the nature of contact between receptors (such as people or ecosystems) and physical, chemical, or biologic stressors. Exposure science strives to create a narrative that captures the spatial and temporal dimensions of exposure events with respect to acute and long-term effects on human populations and ecosystems” (NRC 2012).

Internal and external exposure. Internal and external exposures are two commonly used classes of exposure metrics. Blood or tissue concentrations from biomonitoring studies are relatively direct measures of internal exposure; amounts or concentrations in biofluids leaving the body (breath and urine) are less direct measures. Internal measures can be estimated from the less direct measures when supporting pharmacokinetic data and models are available. Air or media concentrations are external measures of exposure from which internal measures of exposure might be derived if necessary. What exposure metric is considered appropriate depends on the decision context, confidence in the measurement, and proximity to the site of action.

Near-field chemical exposures. Near-field human exposures result from chemical release or use near a person. Near-field chemical exposures include direct dermal application (for example, of sunscreen or cosmetics), direct inhalation (for example, of tobacco smoke or pharmaceuticals), and direct ingestion (for example, of pharmaceuticals). Near-field chemical exposures can also result from the intentional use (as in the case of consumer products) and inadvertent release (as in the case of building materials) of chemicals near a person and later near-field transport to a person that results in contact or intake through inhalation, dermal, or ingestion pathways.

Far-field chemical exposures. Far-field human exposures result from release or use distant from a person. They can also result from initial near-field use (indoors) and later fate and transport in the natural environment (outdoors) before the chemical reaches a person. Far-field exposures can result from inhalation of outdoor air and ingestion of drinking water and foods that contain chemicals that have entered the contact media through fate and transport processes in the natural environment.

Aggregate exposure. Aggregate exposure is exposure to a given substance from multiple sources via multiple pathways and routes (that is, combined exposure from multiple sources by dermal, ingestion, and inhalation routes).

The studies generated with remote sensing data provide even greater insights into human exposures when coupled with GPS and GIS data on populations of interest. GPS data are used to track people in observational exposure and epidemiological studies (Elgethun et al. 2007), and recent advances have allowed more automated coding of GPS data on activities and microenvironments, such as inside and outside at home and at work (Wu et al. 2011; Breen et al. 2014; Nethery et al. 2014; Andra et al. 2015). Data on microenvironments can be used as input for exposure models to refine exposure estimates based on remote sensing data, ground-based ambient air data, and indoor air monitoring data (Breen et al. 2014). Advances in GPS technologies have also been coupled with sensor technologies that measure basic health data, such as heart and respiratory rates and activity level. Information on such measures can be additional inputs for the exposure models and allow further refinement and improvement of exposure classification (Andersen et al. 2015).

Computational Exposure Assessment

For the vast majority of stressors, there are few exposure measurements (Muir and Howard 2006; Egeghy et al. 2012). Various conceptual, empirical, and predictive exposure models are needed to address those data gaps and to enhance the usefulness and application of measured data to exposure and risk as-

assessment. Since the release of the ES21 report, there has been substantial research activity and advancement in the development of computational exposure tools, particularly for calculating near-field chemical exposures of humans, for quantifying relationships between external and internal exposures and between in vivo and in vitro exposures, and for high-throughput exposure estimation that has been used alone and in combination with bioactivity data to set priorities for chemical assessment.

Egeghy et al. (2011) reviewed tools designed to set priorities rapidly for large numbers of chemicals, and Mitchell et al. (2013) conducted an “exposure model prioritization challenge.” A key finding of the challenge was the need to reconcile exposures to chemicals released outdoors (far-field sources) with exposures to chemicals in consumer products applied directly or through indoor-environment exposure pathways (near-field exposures). The recognized absence of tools and exposure information is stimulating research to develop and improve near-field and far-field exposure science. Specifically, the seminal model developed for simulating chemical transport in an indoor environment (Bennett and Furtaw 2004) has been revised to include exposure pathways for which external human exposures (intake fractions) (Shin et al. 2012) and internal exposures (estimates of whole-body concentrations) (Zhang et al. 2014; Webster et al. 2016) can be estimated. Furthermore, data and models are evolving to improve mechanistic understanding of chemical releases and exposures indoors (Weschler and Nazaroff 2010, 2012; Little et al. 2012). Exposure models for consumer products also are evolving and being evaluated for select chemicals (Young et al. 2012; Gosens et al. 2014; Delmaar et al. 2015; Dudzina et al. 2015). Exposure models and frameworks that combine far-field and near-field pathways for aggregate human exposure assessments are also being developed and applied (Isaacs et al. 2014; Shin et al. 2015; Fantke et al. 2016).

EPA’s ExpoCast project conducts research on and uses computational tools for high-throughput exposure estimation or “forecasting” to set testing or assessment priorities. The ExpoCast project combines various models and data sources to estimate exposures, which can then be compared with high-throughput ToxCast data and other sources of toxicity or bioactivity data. As a part of the ExpoCast exposure estimation, the Systematic Empirical Evaluation of Models (SEEM) framework includes calibration and evaluation of exposure-model estimates against chemical concentrations measured in or estimated from blood and urine samples from a group of nonoccupationally exposed US residents over the age of 6 years (Wambaugh et al. 2013, 2014).² Exposure-model predictions are compared with available biomonitoring data to estimate the uncertainty in the combined exposure-model calculations (Wambaugh et al. 2013). The Stochastic Human Exposure and Dose Simulation Model for Multimedia, Multipathway chemicals (SHEDS-MM) for exposure-based priority-setting and screening has been revised for high-throughput capacity (SHEDS-HT) (Isaacs et al. 2014) and feeds into the SEEM framework. Other complementary high-throughput aggregate exposure-estimation models that combine existing and emerging tools (see, for example, Shin et al. 2015) can also be incorporated into the SEEM framework, and they are being applied, evaluated, and refined in other contexts.

Improving the amount and quality of the data that are needed to develop parameters for the computational exposure tools is critically important; without such data, the applicability of the tools is limited. Some advances include updated exposure factors (EPA 2011) and the development of the Consumer Product Chemical Profile Database (Goldsmith et al. 2014) and the Chemical/Product Categories Database (Dionisio et al. 2015).³ Numerous quantitative structure–activity relationship (QSAR) models, quantitative structure–property relationship (QSPR) models, and other computational tools for predicting chemical-property information—such as solubilities, partition coefficients, and degradation rates—continue to evolve. The applicability domains of existing tools for calculating chemical-property information require further examination and more explicit definition to ensure that the models are calibrated and applied within the same chemical space. Integrated testing strategies to obtain more high-quality measurements can then be strategically developed to expand the applicability domains of current QSAR models, QSPR models, and other tools used for property estimation.

²Data are from the US National Health and Nutrition Examination Survey.

³See <http://actor.epa.gov/cpcat>

Because of the extensive measurement-data gaps, the recent advances in computational tools for exposure science are expected to play a crucial role in most aspects of exposure estimation for risk-based assessments, not only high-throughput applications. Higher-tiered models that link exposure databases and spatial information (see, for example, Georgopoulos et al. 2014) and opportunities to combine and integrate measurements and models to characterize and quantify the source-to-receptor relationship more fully (see, for example, McKone et al. 2007) are being developed and applied. Exposure-model uncertainty and sensitivity analyses are useful computational methods that can be used to set priorities for exposure-science research systematically (Arnot et al. 2012; NRC 2012; Arnold et al. 2014).

Personalized Exposure Assessment

Behavior patterns that determine exposure routes, durations, and conditions combined with the variation in environmental concentrations of stressors over space and time result in unique exposure patterns for individuals and populations. Exposure data that are needed to assess personal exposures can now be generated on various spatial and temporal scales with traditional and emerging methods. New opportunities to measure exposures in and outside the body will help to characterize and quantify personal exposures to an array of stressors. Particularly notable are recent advances in the application of passive sampling techniques to determine internal human concentrations (for example, using silicone implants) (Allan et al. 2013a; Gilbert et al. 2015; O'Connell et al. 2015), external exposure concentrations integrated over time and space (for example, using silicone wristbands) (O'Connell et al. 2014a,b), and chemical concentrations and chemical activities⁴ in media to which humans are exposed, such as foods (Allan et al. 2013b; Jahnke et al. 2014) and indoor air (Wetzel and Doucette 2015). Portable sensors for measuring particles and volatile organic chemicals are being refined and are providing valuable information on personal exposures, particularly in vulnerable populations (McGinn et al. 2016). Mobility-based exposure assessment that uses personal devices, such as cell phones, to provide GPS information, can be used to determine time and location of people relative to exposure levels determined from remote sensing information (Adams et al. 2009; de Nazelle et al. 2013; Su et al. 2015). Consumer product and use databases and market research data can provide population and personal exposure information that can help to inform exposure assessment, for example (Goldsmith et al. 2014). All those emerging technologies and data streams will complement existing tools and techniques in the effort to obtain more comprehensive knowledge of the source-to-outcome continuum.

Targeted and Nontargeted Exogenous Chemical Exposure Assessment

Important advances in two complementary approaches for characterizing human exposure—targeted and nontargeted analysis—are improving the accuracy and breadth of human and ecological exposure assessment (Fiehn 2002; Park et al. 2012; O'Connell 2014a,b; Go et al. 2015; Mastrangelo et al. 2015; Sud et al. 2016). Both approaches, whether focused on endogenous or exogenous chemicals, are commonly referred to as metabolomics approaches.⁵ Targeted analysis focuses on selected chemicals for which standards and methods are available and identifies chemicals on the basis of mass spectrum, elution time, detector signals, or some combination of these measures. Targeted analysis has produced much of the ex-

⁴Chemical activity is related to the energetic state of a chemical, is a measure of the *effective concentration* of a chemical in a given exposure medium (Reichenberg and Mayer 2006; Mackay et al. 2011), and is closely related to the freely dissolved concentration. For example, chemical activity is an improved measure of exposure when interaction with media constituents (such as particles in air and organic matter in water) effectively reduces the amount of chemical free to interact with a biological receptor (such as a human), often referred to as the bioavailable fraction.

⁵As defined in Chapter 1 (see Box 1-1), metabolomics is assumed to include exogenous chemicals found in biological systems in their unmetabolized forms.

posure data used in epidemiological studies and risk assessment. The US National Health and Nutrition Examination Survey and the Canadian Health Measures Survey are two extensive biomonitoring programs that use targeted analytical techniques for exposure assessment (Needham et al. 2005; Calafat 2012; Haines and Murray 2012). Although initially limited by throughput and a focus on single chemicals, small groups of chemicals (Casas et al. 2011; Mortensen et al. 2014), or modest-size chemical classes (O’Connell et al. 2014b), targeted methods are emerging for quantitative analysis of hundreds of chemicals (O’Connell et al. 2015). Generally, there is a tradeoff between sensitivity and selectivity that imposes limitations on the number of chemicals that can be analyzed in single runs by using a single instrument or method. Targeted analyses are limited to chemicals for which standards are available. Accepted standards for identification and quantitation have been articulated for most analyte classes (such as metabolites and peptides) (Castle et al. 2006; Fiehn et al. 2006; Goodacre et al. 2007; Sumner et al. 2014), but these standards are inconsistently applied in practice.

Targeted analytical methods for protein and DNA adducts have emerged as an alternative to direct measurement of chemicals in blood. When stable protein or DNA adducts can be easily measured and information on the rates of adduct formation and loss is available, adduct concentrations can be used as proxies for the time-weighted average exposure to the parent chemical. Those approaches are particularly valuable for exposure assessment and exposure reconstruction for short-lived chemicals whose concentrations in blood and other biofluids might be very low and subject to high temporal variability. One example is the use of hemoglobin adducts of acrylamide and its metabolite glycidamide for accurate reconstruction of acrylamide exposure and its concentration in blood over time in humans (Young et al. 2007). Chemical-specific adducts of the carcinogens butadiene, formaldehyde, and acetaldehyde have emerged recently as metrics of exposure to these extremely short-lived chemicals (Swenberg et al. 2007; Swenberg et al. 2008; Moeller et al. 2013; Yu et al. 2015). The benefits of using stable adducts to measure exposure to short-lived chemicals include the ability to integrate exposure over time (that is, the adducts can serve as integrative measures of exposure because they are more stable) and biological relevance because of the proximity to a target site, such as DNA. Swenberg and co-workers have established highly sensitive methods for specific formaldehyde DNA adducts and pioneered methods for establishing the contribution of endogenous and exogenous formaldehyde to total internal exposure (Edrissi et al. 2013; Moeller et al. 2013; Pottenger et al. 2014; Pontel et al. 2015; Yu et al. 2015). The studies highlight the utility of targeted analysis of adducts for exposure assessment and perhaps a potential for broad assessment of the adductome (Gavina et al. 2014; Pottenger et al. 2014).

Nontargeted analysis has emerged as an approach to provide qualitative information on the large portion of the exposome that is uncharacterized—a portion that includes bioactive endogenous peptides, exogenous chemicals, metabolites, lipids, and other biomolecules. It offers the ability to survey more broadly the presence of all chemicals in the environment and in biofluids regardless of whether standards and methods are available. The nontargeted approach trades selectivity for breadth and produces numerous unidentified analytical features. Comparing unidentified analytical features from large cohorts and correlating them with responses of interest in the cohorts can help to identify analytical features for further investigation (Burgess et al. 2015). Cheminformatics and computational chemistry can be used to identify chemicals with varying levels of confidence; nuclear magnetic resonance spectroscopy can be used to identify chemical structure with high accuracy. Accepted standards for identification of metabolites (Castle et al. 2006; Fiehn et al. 2006; Sumner et al. 2014) have not been routinely applied to nontargeted approaches, so chemical matches to the analytical features is tentative and association between specific chemicals and disease is uncertain.

Nontargeted approaches are promising, but there are limitations in the use of data produced from nontargeted analyses that should be considered before collecting the data. For example, an unidentified analyte cannot be used to develop a mechanistic argument to support or refute a causal association between the presence of the analyte and a clinical effect, it cannot be quantified in absolute terms, it cannot be subjected to toxicity testing, and it cannot be attributed to sources for purposes of exposure mitigation.

Although identifying all analytes is an important objective, reducing the number of analytes—to investigate, for example, on the basis of frequency in samples, membership in an important chemical class, and association with a clinical outcome—will be important until methods for identification of unknown analytes become more efficient.

Initial efforts to understand potential contributions of exogenous and endogenous exposure have led to important insights about the role of each and about potential limitations of analytical technologies. Rappaport and co-workers (2014) reported human blood concentrations of many chemicals, their sources, evidence of chronic-disease risks, and numbers of metabolic pathways. Blood concentrations of endogenous chemicals, food chemicals, and drugs were indistinguishable and spanned 11 orders of magnitude; blood concentrations of pollutants were on the average lower by a factor of about 1,000 (Figure 2-2). Although the findings cannot be generalized to all chemicals or all exposure scenarios, the blood-concentration ranges highlight the importance of using highly sensitive analytical instrumentation to characterize human exposure (Athersuch 2016; Uppal et al. in press).

Risk assessment and mitigation of sources and risks all depend on knowing absolute quantities of specific chemicals; therefore, targeted analyses will continue to be the primary source of exposure information. Because the results of nontargeted analyses provide only relative or qualitative exposures, they are not readily applicable to conventional risk assessment. However, when unidentified analytical features can be aggregated according to their toxicity or pharmacokinetic behavior, there will be new opportunities to conduct hazard or risk assessments on the basis of similarity to chemicals whose toxicity is known.

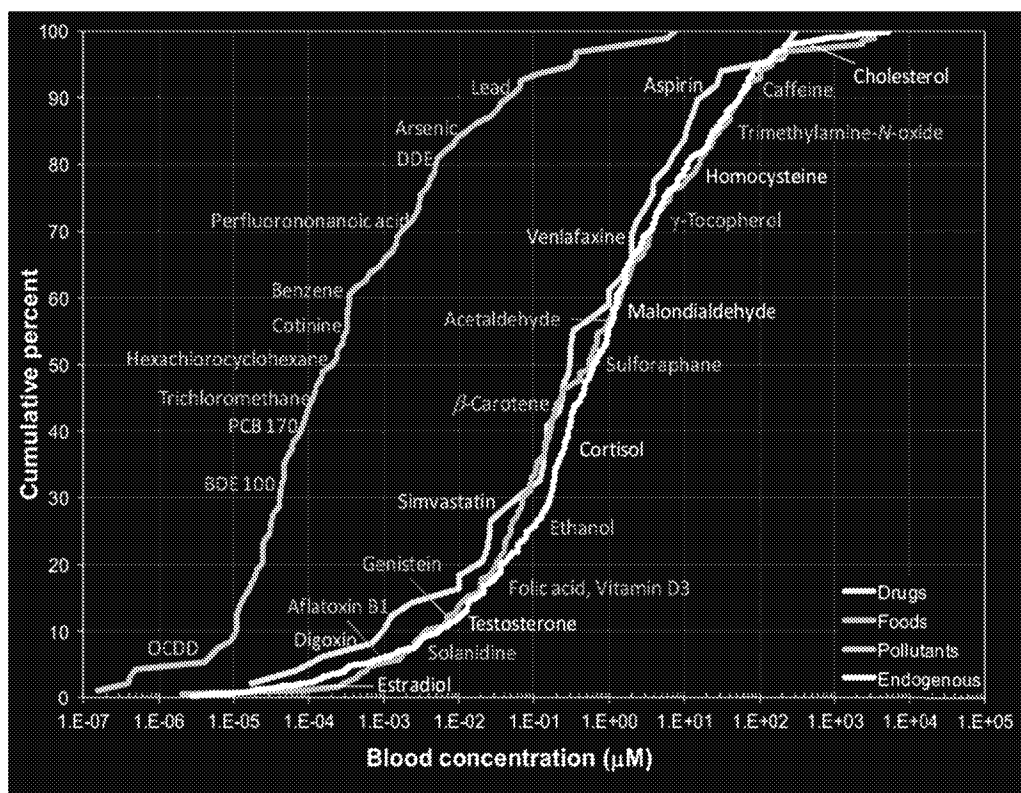


FIGURE 2-2 A survey of measured blood concentrations shows that for the selected chemicals concentrations of pharmaceuticals and naturally present endogenous chemicals are similar and are generally higher than concentrations of environmental contaminants. The findings highlight the importance of using highly sensitive analytical instrumentation to characterize human exposure. Source: Rappaport et al. 2014.

Exposure Inference from -Omics Technologies

-Omics technologies that quantify the abundance of biomolecules, such as proteins and transcripts, offer distinct and diverse applications for exposure assessment. In contrast with metabolomic approaches that quantify exposure to specific metabolites of endogenous and exogenous chemicals, proteomic and transcriptomic approaches provide global assessment of biological responses to exposure to multiple stressors. Those -omics approaches can provide biomarkers or biosignatures of response to chemical classes, such as oxidants (Roede et al. 2013; Go and Jones 2014) and potentially genotoxic chemicals (Fenech and Bonassi 2011; Lovreglio et al. 2014; Kalembe-Drozdz 2015; Moro et al. 2015; Tumer et al. in press). That particular application of -omics technologies, a key element of Wild's original vision of the exposome (Wild 2005, 2012), is used to *infer* exposure to one or more chemicals on the basis of a mechanistic understanding of biological response to them. Some biomarkers of exposure can result from changes in the body that are induced by chemical exposure (for example, changes in metabolite or protein profiles), but these types of biomarkers commonly do not provide quantitative exposure information that can be used for risk estimation. The application of -omics technologies to infer exposure to classes of stressors is expected to grow. Although the initial utility will probably be in qualitative exposure inference and in assembling evidence on biological pathways, application should expand to more confident and more quantitative characterization of exposures to chemical classes or groups of stressors that produce the same biological effect, such as oxidation or inflammation.

Novel Exposure Matrices for Exposure Reconstruction

Assessment of occupational and environmental exposures will continue to rely on matrices for which there are established methods of collection, analysis, and interpretation. Those matrices include air, water, soil, food, blood, and urine. The expanding computational exposure-science infrastructure (Arnot et al. 2012; Shin et al. 2012, 2015; Wambaugh et al. 2013, 2014; Isaacs et al. 2014), which uses the traditional data streams to construct population-level exposure assessments, will continue to drive the generation of data on the traditional exposure matrices.

Growing emphasis on near-field exposures (Stapleton et al. 2008; Shin et al. 2012; Wambaugh et al. 2014) and on exposures during development, which is the focus of the Children's Health Exposure Resource Centers of the National Institute for Environmental Health Sciences, is poised to drive exposure assessment toward new environmental and biological matrices and new approaches. For example, population-level exposure to hundreds of chemicals was recently shown to be dominated by near-field exposures from consumer-product and household use, not by far-field exposures that take place after chemicals are released into the outdoor environment (Shin et al. 2012; Wambaugh et al. 2014). Increased focus on categorizing chemicals in consumer products and on assembling exposure data for use in exposure assessment is one immediate outcome of the recent studies. Continued efforts to measure and estimate concentrations in multimedia sources—such as indoor air, indoor surfaces, dust, and consumer products—are required to address uncertainty in near-field exposures and pathways.

Characterization of exposures during the toxicologically sensitive period of fetal development has historically been limited to drawing inferences about maternal exposure through periodic maternal blood and urine measurements. Responding to the need to improve the characterization of fetal exposures to chemicals, researchers have turned to novel biological matrices, such as teeth, hair, nails, placental tissue, and meconium. The growth properties (the sequential deposition or addition of tissue) and availability of these biospecimens offer the opportunity to extract a record of exposure. For example, laser-ablation inductively coupled mass spectrometry has been used to reconstruct the timing of shifts in primates' diets that are associated with weaning by measuring calcium:barium ratios in tooth enamel (Austin et al. 2013). The same approach was recently shown to be promising for assessing in utero exposure to manganese. Arora et al. (2012) measured manganese concentrations in tooth dentine specific to the postnatal period and the second and third trimesters and showed a statistically significant relationship between house-dust

manganese concentrations and dentine manganese concentrations during the second trimester. Those authors and others (Andra et al. 2015; Palmer et al. 2015) have extended the methods to measure organic chemicals, including phenols and phthalates. Like teeth, hair forms in utero (third trimester), continues to grow, and potentially provides a temporal record of exposure. Initially used widely for forensic analysis of exposure to illicit drugs, hair has emerged as an important matrix for biomonitoring of metals and organic chemicals, such as polybrominated diphenyl ethers (Aleksa et al. 2012; Liu et al. 2015a). Similar methods have been applied to fingernails (Liu et al. 2015a).

Although the new matrices mentioned above have advantages and add valuable information to exposure assessment, they pose challenges in interpretation and application. A common challenge in the use of exposure measures based on the new biological and environmental matrices for quantitative exposure assessment is the need to understand how measured concentrations are related to measures of exposure traditionally used to assess chemical toxicity or risk. Ideally, the new biomonitoring data can be supported by information regarding how measured concentrations in new matrices are related to conventional measures of internal exposure (serum concentrations, μM) or external exposures (mg/kg-day or mmol/kg-day). New experimental data, such as chemical half-life in the body, and data related to events and processes of exposure, such as time since the exposure, that can inform various relationships and pharmacokinetic models will be useful in interpreting and reconstructing exposures by using the biomonitoring data (see, for example, Lorber and Egeghy 2011; Ritter et al. 2011; Quinn and Wania 2012; Wambaugh et al. 2013; Aylward et al. 2014; Hays et al. 2015). The additional information regarding the exposures provides confidence in using the measured biomonitoring data and supporting the exposure narrative.

Physiologically Based Pharmacokinetic Models and Models for Translating Exposure Between Systems

Physiologically based pharmacokinetic (PBPK) models have made substantial contributions to exposure assessment for more than 30 years. PBPK models have been applied effectively to characterize target-tissue exposure in test animals and humans, to characterize pharmacokinetic variability, and to extrapolate across species, life stages, exposure routes, and, most recently, ecosystem elements (MacLachlan 2010; Weijs et al. 2012; Sonne et al. 2015). PBPK models now provide a common framework similar to environmental fate and transport models for more integrative exposure assessment and are applied more regularly to support aggregate (multiroute) exposure assessment (Esch et al. 2011; Abaci and Shuler 2015), exposure reconstruction from biomonitoring data, and exposure translation between in vitro and in vivo test systems.

The use of PBPK models for exposure reconstruction, known as reverse dosimetry (Liao et al. 2007; Tan et al. 2007; Bartels et al. 2012; Hays et al. 2012; McNally et al. 2012; Yang et al. 2012; Grulke et al. 2013), has led to important advances in the field of biomonitoring. Internal and external exposures can now be related and predicted on the basis of more limited sets of exposure information—for example, urine biomonitoring data (spot samples)—by applying principles of pharmacokinetics. The tools are used to calculate or estimate margins of exposure to chemicals on the basis of blood or urine spot samples and can be used to inform regulatory levels. New methods offer the ability to evaluate the influence of behavior and physiological variability on exposure distributions (Shankaran and Teeguarden 2014).

The use of PBPK models to characterize the influence of biochemical and physiological variability, particularly the role of polymorphisms of metabolizing enzymes in estimates of metabolism and variability (Beaudouin et al. 2010; Bois et al. 2010; Snoeys et al. 2016), has grown substantially and will continue to contribute to exposure assessment and risk assessment. Those advances help to predict pharmacokinetics of potentially sensitive populations, such as preterm infants (Claassen et al. 2015) and children (Yoon et al. 2012). Recently, PBPK models have been applied to disentangle the role of physiological changes related to disease states from the effects of a chemical on disease and to examine the role of reverse causation in published epidemiological studies (Verner et al. 2015; Wu et al. 2015). Accordingly,

PBPK models have emerged as new exposure tools capable of supporting inference in epidemiological studies.

One of the major developments concerning PBPK models has been their use for translating exposures between test systems and human-exposure scenarios. In particular, the rapidly expanding use of high-throughput in vitro cell and cell-free systems to characterize the bioactivity of chemicals and materials, such as nanomaterials, has led to a need to translate in vitro exposure data into corresponding in vivo exposures in test systems and humans. Various terms have emerged to describe the applications to do so—for example, in vitro–in vivo extrapolation (IVIVE), reverse toxicokinetics (rTK), and reverse dosimetry. Each describes a kinetics-based and partitioning-based approach to translating exposures from one system of interest (in vitro) to another (in vivo animal or human), and all strive for mass balance. The use of PBPK models and similar biokinetic models of in vitro test systems has produced important methods that can apply PBPK-modeling principles to a broad set of test systems (Rostami-Hodjegan 2012; Yeo et al. 2013; Campbell et al. 2014; Teeguarden et al. 2014; Martin et al. 2015), including microphysiological organ systems or human-on-a-chip systems (Esch et al. 2011; Abaci and Shuler 2015). However, without clear understanding of how exposures in the systems are related to in vivo exposures or human occupational or environmental exposures, their utility will remain limited, as has been the case for standard in vitro cell-culture and cell-free systems.

IVIVE models can be used to calculate human internal exposure concentrations of chemicals from data obtained in high-throughput in vitro systems (Kesisoglou et al. 2015). That approach uses hepatocyte cultures and other biotransformation systems to measure metabolic rate constants that are used to estimate human intrinsic clearance by the liver, a dominant route of metabolic and total clearance in humans. Clearance values can be obtained for different life stages or for populations that are resistant or vulnerable because of polymorphisms of metabolic enzymes. Renal clearance, another major elimination pathway, is often estimated by using data on glomerular filtration rates and measures of protein binding in serum (Rule et al. 2004; Rotroff et al. 2010; Tonnelier et al. 2012; Wetmore et al. 2012). Other aspects of kidney function, such as tubular processing, can also influence clearance rates (Weaver et al. 2016) and various biomarker concentrations. Metabolism in other tissues, which can be important, is not evaluated, and this is a limitation of the current state of these systems.⁶ Combining clearance with computational high-throughput methods for estimating average daily contact and intake rates makes it possible to predict internal concentrations expected in humans. Those concentrations can then be compared with effect levels or no-effect levels from toxicity-testing systems. Addressing some limitations—such as not accounting for metabolism by other tissues, for the potential role of transporters, or for human variability—will be important next steps toward higher confidence in the application of the models. New approaches for better understanding of metabolic and genetic determinants of exposure are detailed in the next section.

Key challenges in interpreting and applying IVIVE data include the quantification of relevant concentrations that correspond to observed in vitro bioactivity from assumed nominal (administered) concentrations (see Box 2-2 and Figure 2-3). A consistent approach for comparing and extrapolating results could be the use of the free (dissolved aqueous) concentration in the test system because this metric can be applied to cell-based or cell-free systems. The limitations complicate chemical comparisons for potency and toxicity and reduce confidence in the application of in vitro bioassay data that are based only on nominal concentrations in risk-based assessments. Models to calculate in vitro concentrations that cannot be readily measured with traditional sample extraction and analytical techniques need to be developed, evaluated, and applied. Passive dosing and sampling techniques might prove useful in addressing the current analytical challenges and associated uncertainties in quantifying exposures in smaller in vitro test systems (Kramer et al. 2010).

⁶The committee notes that over-prediction of serum concentrations of parent chemicals and under-prediction of potentially important metabolites is generally a possible outcome of underrepresenting metabolism.

BOX 2-2 Challenges in Estimating In Vitro Test Concentrations

Evidence is accumulating that the prevailing view that stressor concentrations in the in vitro systems can be considered static and can be represented by nominal media concentrations is in many cases not valid (Gulden and Seibert 2003; Gulden et al. 2006; Teeguarden et al. 2007; Kramer et al. 2012; Armitage et al. 2014; Teeguarden et al. 2014; Groothuis et al. 2015). For example, nanomaterials, an emerging class of poorly studied toxicants, undergo transformations (agglomeration and dissolution) in liquid systems and size-dependent and density-dependent diffusion and sedimentation; each process affects delivery of particles to cells in culture. The processes have been shown repeatedly to affect cellular dose and can be expected to affect relative hazard ranking. Chemical concentrations in an in vitro test system can change as a function of the chemical properties, the test system, and time. Measured and estimated dissolved and cell concentrations can be orders of magnitude different from assumed (nominal) in vitro concentrations for various reasons, including chemical volatilization, differential distribution in the test system (Heringa et al. 2004; Kramer et al. 2012; Armitage et al. 2014), metabolism (Coecke et al. 2006; Groothuis et al. 2015; Wilk-Zasadna et al. 2015), and the reasons noted above.

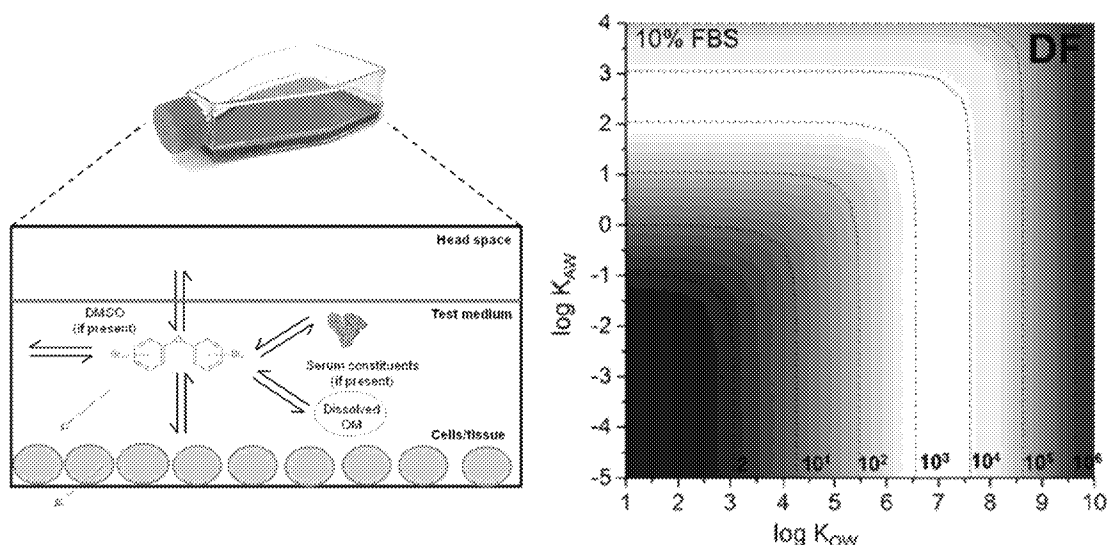


FIGURE 2-3 (Left) Illustration of chemical distribution in an in vitro test system and (right) illustration of the chemical depletion factor ($DF = C_{\text{nominal}}/C_{\text{dissolved}}$) in a typical cell-based in vitro test system as a function of chemical partitioning properties. The octanol–water partition coefficient (K_{OW}) characterizes chemical partitioning from water to nonaqueous constituents of the test system—such as cell membranes, proteins, plastic, and serum—and the air–water partition coefficient (K_{AW}) characterizes chemical partitioning from water into air or head space. In this case, 10% fetal bovine serum (FBS) is assumed present in the test system. The dotted lines (right) are the DFs corresponding to the chemical-property combinations and indicate the order-of-magnitude differences that can occur between assumed (administered or nominal) test concentrations typically used for dose–response calculations and the estimated dissolved (free) concentration in the test system. Source: Armitage et al. 2014.

New Approaches for Assessing Biochemical and Physiological Determinants of Internal Exposure

Metabolism, cellular transport, and other processes that control elimination and distribution of chemicals in organisms are essential considerations and important challenges in exposure science, data interpretation, and risk assessment. Metabolism is a key determinant of chemical residence time in the body and can lead to more or less production of toxic chemicals; thus, it plays an important role in the extent of exposure and chemical toxicity (Leung et al. 2012). Reliable measures of metabolic rates are

essential for understanding and characterizing differences in metabolism among species and between in vitro and in vivo test systems and for understanding the extent of variability and its effect on susceptibility or resistance. Computational approaches (PBPK, rTK, and IVIVE) can be used to translate in vitro metabolic rates into estimates of chemical clearance (Wilk-Zasadna et al. 2015) and to quantify differences among species and systems for exposure assessment.

High-throughput in vitro assays can be used to investigate metabolism; they now cover many enzymes and isoforms involved in chemical metabolism, including the phase I cytochrome P450 enzymes and a variety of phase II enzymes (admescope; Tolonen and Pelkonen 2015). Direct measures of activity obtained from the assays complement genomic approaches for characterizing the influence of polymorphisms on metabolism. New proteomic tools that use chemical probes can also be used to measure metabolic activity of specific enzymes directly in tissue and cellular preparations (Cravatt et al. 2008; Sadler and Wright 2015). For example, recent publications (Crowell et al. 2013; Sadler et al. 2016) demonstrate that activity-based probes provide better measures of relative enzyme activity for individual enzymes than measures of transcripts or proteins and thus complement conventional metabolism assays. Other in vitro metabolism test systems, such as ones that use hepatocytes and liver spheroids, and computational models to translate metabolic rates and pathways to in vivo clearance continue to evolve (Fitzgerald et al. 2015; Hutzler et al. 2015; Liu et al. 2015b). Higher-throughput systems for measuring and interpreting metabolic rates in hepatocytes have been successful in extending our knowledge from pharmaceuticals to environmental chemicals (Wetmore et al. 2014; Yoon et al. 2014). However, increasing capacity to synthesize chemical standards and test materials will be essential if these approaches are to be successfully applied to the many chemicals in commerce.

As basic hepatic-metabolism data grow, other limitations of the systems to predict chemical kinetics and internal exposures will become important. Extrahepatic metabolism—such as metabolism in the kidney, gastrointestinal tract, and lung—can be important but is not yet addressed in most extrapolations. Similarly, differences in metabolic competence between the cells used in vitro and the in vivo systems can affect the extent of metabolism, the metabolic pathways activated, and the metabolites produced (see, for example, Kolanczyk et al. 2012). Emerging tools that can evaluate potential metabolite production (Tolonen and Pelkonen 2015; Wilk-Zasadna et al. 2015) and the use of multiple in vitro metabolism systems of varied complexity (Zhang et al. 2012) that include more than one tissue or cell type are possible solutions to the challenges. QSAR models that can predict rates of metabolism and clearance in tissues, such as liver and plasma (Berellini et al. 2012; Hsiao et al. 2013), and in the whole body (Obach et al. 2008; Wishart et al. 2008; Arnot et al. 2014) are also promising approaches for obtaining information on metabolism.

Pharmacogenomic profiling has emerged as a valuable approach for characterizing individual and population variabilities in genes that influence absorption, distribution, metabolism, and elimination (ADME) of drugs and environmental chemicals. Variations in ADME processes are important sources of variability in internal exposure. Recent advances in sequencing technologies (De Wit et al. 2015; Heather and Chain 2015; McGinn et al. 2016) now offer unprecedented potential for rapid individual and population-level identification of single-nucleotide polymorphisms that affect metabolic, transport, and clearance processes that together influence individual internal-exposure profiles. Recently, the frequencies of polymorphisms in 1,936 proteins that have documented clinical significance for ADME processes were measured and characterized in a Thai population and compared with findings in other ethnicities (Jittikoon et al. 2016). That and other recent analyses that show greater diversity in polymorphisms in American blacks and other ethnicities (Li et al. 2014; Ortega and Meyers 2014) demonstrate the potential for nearly comprehensive assessment of polymorphisms of ADME-related genes in individuals and populations and for internal-exposure predictions on an individual basis. More comprehensive characterization of ADME-related and other polymorphisms in populations and improved understanding of their function and relevance to exposure and toxicity will be valuable in addressing population variability for risk-based decision-making. The committee notes that compartmental and PBPK models for predicting the resulting effects on population distributions of serum concentrations have been used regularly but for only a few metabolic enzymes (EPA 2010).

Another important process to consider is cellular transport; transport proteins influence both tissue and intracellular concentrations. Pharmaceuticals and environmental chemicals are substrates for transporters (Fardell et al. 2011), and the importance of transporters in affecting internal chemical exposure at target sites is recognized (Wambaugh et al. 2014). QSAR models for predicting chemical interactions with transporters (Sedykh et al. 2013) and a variety of in vitro assays (Xie 2008) have been developed to support incorporation of transporters into determinations of internal exposure.

Continued success in using the new tools described here for measuring and calculating biochemical and physiological determinants of internal exposure will improve exposure assessment and ultimately will support the successful integration of in vitro, computational, and in vivo approaches into risk assessment.

CONFIDENCE LEVELS IN EXPOSURE INFORMATION AND ASSESSMENT

Exposure data from traditional and emerging methods discussed above can be placed in categories spanning the continuum from source to target-site exposure (Figure 2-4) (NRC 2012). Exposure measures biologically closer to the site of action of the stressor can under some conditions have greater value for linking exposures to effects. For example, the relationship between soil concentrations of a chemical and effects in a population exposed to the soil might be obscured by individual differences in exposure rate, activity patterns, and metabolism. In contrast, individual blood or tissue measures of chemical exposure reflect the combined action of those processes and benefit from being more directly related to the event that initiates adverse effects: interaction of the chemical with a biological receptor (organelle, protein receptor, or DNA). However, soil and air measures of chemicals and biologics can be less confounded sources of information for assessing source contributions to external exposure because fewer processes (absorption, metabolism, and human activity patterns) can obscure relationships between the measured exposure in blood or urine and the source. The committee cautions, however, that internal exposures are not universally better or universally more useful than external exposures for purposes of relating exposures and effects, for example, in epidemiological studies. A long history shows the utility of measures of external exposure for epidemiology. In fact, external exposures might sometimes be superior to internal exposures, for example, when the two are proportional to one another and external measures are easier to acquire. Furthermore, external exposures might be the most biologically relevant when portal-of-entry effects, such as skin sensitization, are the focus. Exposure measures should be carefully selected by considering the strengths and limitations of external and internal measures of exposure and the purpose for which they will be used. Ideally, exposure data are available across the entire spectrum illustrated in Figure 2-4, and approaches for connecting them quantitatively have been developed to enable the use of exposures at any point on the continuum.

There is a spectrum of quality of exposure data from screening-level assessments based on limited information to multiroute, multisource exposure assessments to population-scale longitudinal exposure assessments that use validated exposure biomarkers. Important considerations for the application of exposure data in decision-making are the quality of the data and the context in which the data will be used; data quality can be determined by evaluating accuracy, integrity, suitability, transparency, and concordance of multiple lines of data or evidence (WHO 2016). The degree of confidence that is required for exposure data or exposure assessment is balanced with the cost of data acquisition and determined by the decision context established in problem formulation. In some cases, screening-level exposure data that have greater uncertainty might have sufficient accuracy to support important screening-level decisions made by regulatory agencies and might provide the most cost-effective approach (WHO 2016; Wambaugh et al. 2013, 2014). In those cases, transparency is essential for providing understanding and confidence in decisions that stem from exposure assessment; transparency can be obtained by carefully documenting and reporting data quality, suitability, and integrity (WHO 2016). The use of computationally derived exposure estimates that are based on sparse data is an example of possible applications. That approach might be used to make initial decisions to set priorities among stressors for improved exposure assessment, toxicity assessment, or epidemiological assessment. The same data might also be useful for

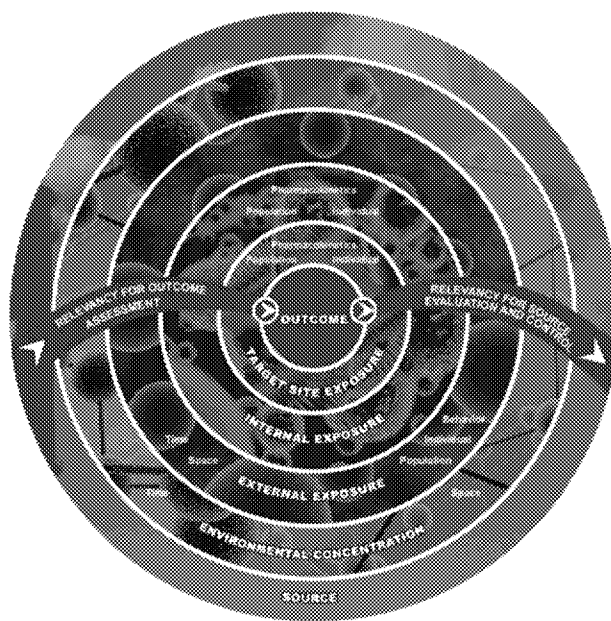


FIGURE 2-4 Exposure measurements are made along multiple points in the source-to-outcome continuum. The value of exposure data for applications, such as source assessment and mitigation and assessment of public-health effects, might depend on location on the source-to-outcome continuum. Careful consideration should be given to selection of exposure measures by balancing cost, invasiveness, and relevance for the study. For example, although internal exposures might be directly related to the event that initiates adverse effects, external measures of exposure might be more relevant to portal-of-entry effects and have the benefit of being more cost-effective to collect. Source: NRC 2012.

making initial decisions regarding new applications of a chemical or its inclusion in or removal from new or existing products. In some cases, extensive uncertainty, sensitivity, and variability analyses of exposure-assessment components might indicate that exposures of the magnitude necessary to cause effects fall outside the range of plausibility, in which case such exposure estimates might have sufficient certainty to support decision-making regarding potential risks. As the field moves toward obtaining exposure data on thousands of chemicals in commerce and wider use of cost-effective screening-level analyses, careful reporting of the quality of assessments and associated limitations—for example, through model evaluation and sensitivity analysis—will have high priority. As computational exposure-measurement tools are developed and used, their successful application in risk-based or exposure-based decision-making as described above will involve passing the same quality assessments applied to environmental measures of exposure, for example, by applying EPA or World Health Organization (WHO) guidance to evaluate models (WHO 2005; EPA 2009, 2016a).

Guidance for evaluating exposure data and exposure assessments developed by WHO and EPA and published in the literature focuses more on determining data quality than on establishing confidence in integrating various data streams. For example, integrating emerging data streams (such as computational exposure data) with conventional data (such as those derived from blood and urine biomonitoring and air sampling) is not discussed. Figure 2-5 presents some general considerations for assessing quality of exposure data and for integrating multiple data types. The four attributes for judging the quality of exposure data outlined by WHO—appropriateness, accuracy, integrity and transparency—also apply to Figure 2-5, but there is additional consideration of the strength of agreement between measures and of how each measure is related to the others in the overall exposure narrative. Although computationally derived exposure estimates might be perceived as warranting less confidence than direct measures, consideration of factors related to appropriateness and accuracy might indicate that the computational estimates are of higher quality. For example, direct exposure measures that are made with analytical methods that have

not been validated, that are confounded by sample contamination, that are determined without accounting for external-exposure intake rates and half-lives, or that lack temporal resolution necessary for their application in some decision-making contexts might ultimately be less valuable than indirect or proxy measures that are based on a validated exposure metric. Similarly, computationally derived exposure estimates might be useful for some decision-making contexts, particularly when they are based on extensive experimental data—including pharmacokinetics, total external exposure, and patterns of external exposure—and show mass balance throughout the system. Confidence in any exposure assessment is increased when there is concordance, consistency, or agreement between multiple methods of exposure assessment and is greatest when directly measured exposures, indirect measures of exposure, and computationally derived exposure estimates or simulations agree (McKone et al. 2007; Cowan-Ellsberry et al. 2009; Mackay et al. 2011; Ritter et al. 2011; Teeguarden et al. 2013). Agreement between measured and predicted data streams builds confidence in each method of determination. Convergence between exposure measurements (external and internal) and model simulation results (for example, overlap of concentrations or probability distributions of concentrations) indicate higher confidence in an exposure estimate and in resulting risk-based decisions. Although agreement between exposure measures might be a hallmark of quality and of the ideal, multiple concordant measures of exposure are not required to establish levels of quality required for all decision-making contexts.

Consideration of the level of quality and confidence in exposure assessment in the decision-making context will continue to be important, particularly as new exposure data streams emerge from personal sampling data and from use of new exposure matrices, such as bone, teeth, and hair. The potential for using emerging exposure data streams is high, but without careful evaluation, comparison with other types of exposure-assessment data, and a consistent effort to relate measurements to the appropriate level of biological organization (for example, target site or source), confidence in their use or agreement on their best application might be difficult to obtain.

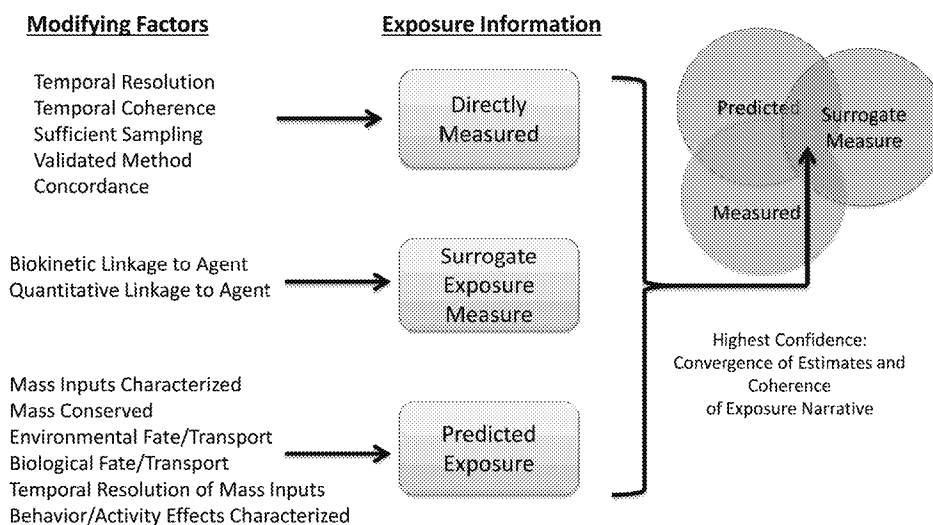


FIGURE 2-5 Confidence increases with more complete characterization of the exposure pathway and associated exposure determinants. Confidence might be higher for direct measures of the stressor—for example, at the site of action—but if such measures do not consider important modifying factors, confidence might be higher for surrogate exposure measures or predicted exposure measures that do consider such factors. The greatest confidence occurs when there is concordance between multiple exposure-estimation approaches or between multiple exposure measures, especially when divergent exposure metrics are considered. The confidence that is required for exposure data and assessments should be determined by data-acquisition costs and the decision context; the highest levels of confidence are not required for many decision contexts.

Guidance has been developed to foster confidence, transparency, and reproducibility in calculated data used for exposure and risk assessment. Specific guidance has been developed for QSAR models for predicting chemical properties and toxicity (OECD 2007), for environmental fate and exposure models (EPA 2009; Buser et al. 2012), and for pharmacokinetic models (McLanahan et al. 2012). As new exposure metrics emerge, it will be important to develop guidance for integrating the various exposure measures and to understand their value and relationships with each other.

APPLICATIONS FOR EXPOSURE SCIENCE

To provide practical guidance on the use of emerging exposure-science data streams for decision-making, the following sections describe applications expected to have near-term and lasting influence on exposure assessment and on risk-based decision-making (Box 2-3). Each application uses one or more of the advances presented earlier in this chapter to provide a new basis for decision-making, to refine exposure data, or to provide new forms of exposure data.

Aligning Exposures Between Test Systems and Humans

Comparison of biological responses across diverse experimental systems is nearly always an essential step in risk assessment. For example, risk assessors are faced with aligning toxicity data that are based on disparate measures of exposure: nominal liquid concentrations or cell concentrations in *in vitro* systems and air concentrations, inhaled amounts, or administered doses in rodent studies and human biomonitoring studies. Specificity, sensitivity, and concordance of observed effects across the test systems underlie the value and strength of evidence supporting conclusions about hazard and risk associated with exposure. To compare the responses from different test systems adequately, the exposures (concentrations) need to be expressed in consistent (comparable) units and with due consideration for the matrix in which the chemical is present. For example, a chemical concentration in whole blood that corresponds to an *in vivo* response can differ from the total concentration in an *in vitro* test system that corresponds to a related response, although the free (dissolved) concentrations in the aqueous phases in each system might be equal. Thus, the alignment of exposures in the systems is one important step in comparing exposure-response relationships across systems and evaluating concordance and consistency. As *in vitro* systems, organotypic, or co-culture systems augment or replace traditional animal studies, biological effects are compared over a more diverse array of assay systems and, from an exposure standpoint, over more types of exposure. For example, the most biologically sound comparison of biological effects shown in a cell-free assay, a cell-based assay, and an inhalation-exposure rodent study would involve comparisons of target-site exposures across all three systems: free-liquid concentrations in the cell-free assay, free cell concentrations in the cell-based assay, and free cell concentrations in the target cells of the rodent. As a practical matter, measured free-liquid concentrations in the *in vitro* assays and serum concentrations in rodent assays or from human studies would typically be considered appropriate measures of exposure-based

BOX 2-3 High-Value Applications for Exposure Sciences

- Aligning exposures between test systems and humans
- Improving exposure assessment for epidemiological studies
- Exposure-based screening and priority-setting
- Identifying new chemical exposures for toxicity testing
- Predicting exposure to support registration and use of new chemicals
- Identifying, evaluating, and mitigating sources of exposure
- Assessing cumulative exposure and exposure to mixtures

alignment of the biological effects. However, there are circumstances in which serum concentrations are not good surrogates for tissue dose—for example, when transport proteins facilitate the uptake to and efflux from the tissue (Koch and Brouwer 2012; Wambaugh et al. 2014). The committee emphasizes that for any metric used to align exposure concentrations between systems, one should consider system conditions that might influence the value or interpretation of the data. For example, is the chemical concentration determined under steady-state or dynamic conditions or is the chemical ionic, in which case pH must be considered?

Each experimental system and human exposure situation has a unique set of processes that control or influence the timing, duration, and extent of exposure at the site of action (see Figure 2-6). Many of the processes are biokinetic and measurable with conventional approaches. Characterizing the processes in each test system allows the measurement, calculation, or simulation of chemical exposure at a common site of action. Consistent metrics of exposure, such as free or cell concentration, represent a possible ideal for comparison across systems and do not have the limitations associated with nominal concentrations. The chemical-activity approach has been proposed for ecological risk assessment (Mackay et al. 2011; Gobas et al. 2015) because it can integrate various multimedia exposure data streams (measured and predicted) and toxicity data streams (in vitro and in vivo) into a framework with consistent units and might be useful for human health evaluations. Other exposure metrics might be suitable for some decision contexts if they are adequately justified on the basis of pharmacokinetics, physical chemistry, and biology of the end point of interest.

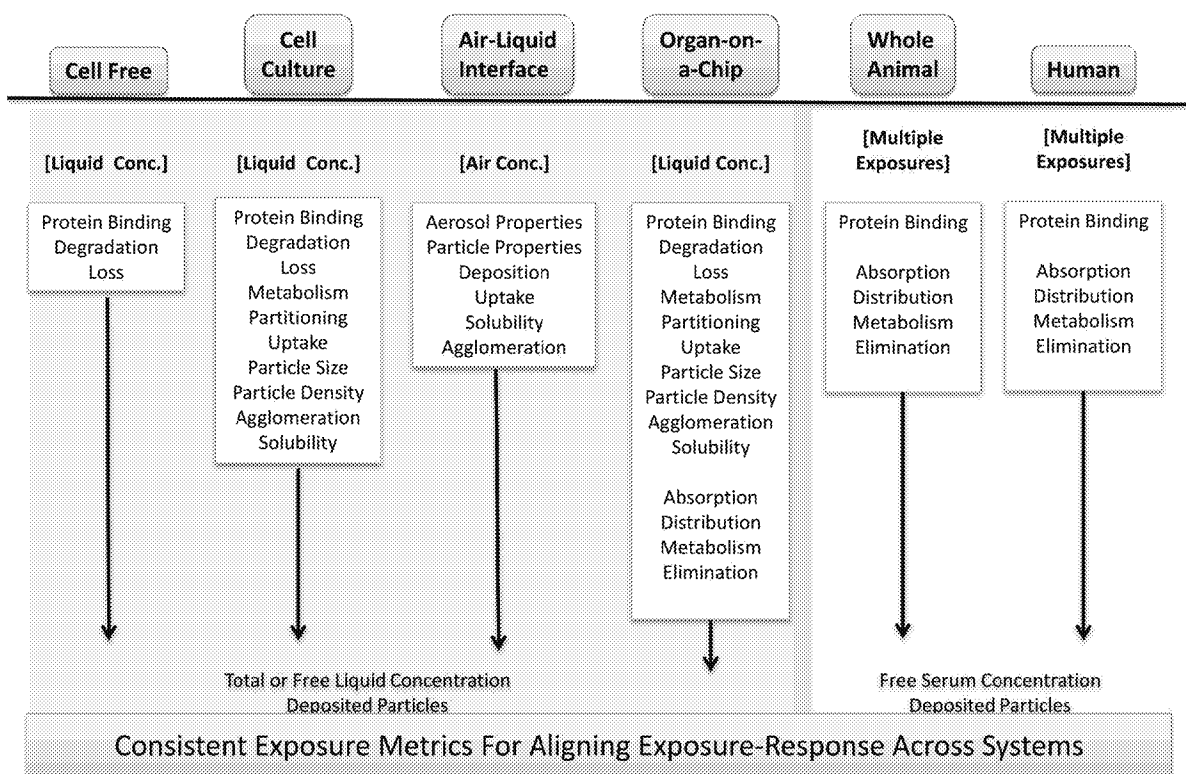


FIGURE 2-6 Alignment of exposures across experimental toxicity-testing systems can be achieved by understanding, measuring, and applying this information on the processes that control the time course of concentrations and delivery of chemicals and particles to target cells in each system. Common target-cell exposure metrics could be total or free concentrations, peak concentrations, or area under the concentration–time curve.

Alignment of exposures between systems can be completed under data-poor and data-rich conditions. High-throughput methods for estimating hepatic and renal clearance can provide data needed for estimating human serum concentrations of chemicals that can be compared with cell-culture concentrations. That approach reflects one extreme—the data-poor case—for which data limitations can be overcome by focused, efficient *in vitro* and computational methods. Recently, an example of alignment of exposures under data-rich conditions—those with data from *in vitro* assays, whole-animal studies, and human biomonitoring—was published for systemic effects. Human urine and serum time-course concentration data from multiple studies provided empirical pharmacokinetic data that showed a relationship between serum bisphenol A (BPA) concentrations and urine BPA concentrations (Teeguarden et al. 2011, 2015; Thayer et al. 2015). The empirical relationships were used to calculate the range of human serum concentrations expected in a population of more than 28,000 people on whom there were published biomonitoring urine data. The resulting range of serum concentrations was compared directly with liquid concentrations in low-dose BPA cell-culture and aquatic studies (Teeguarden et al. 2013, 2015). Conclusions concerning the probability of biological effects in humans were drawn by aligning exposures across human biomonitoring and two divergent test systems—vertebrates and cell-culture systems—that used a measure of exposure proximal to target-tissue exposure. Although the role of protein binding was not addressed in that example, the data and tools to do so for BPA and other estrogens have been developed for rodent test systems and humans (Plowchalk and Teeguarden 2002; Teeguarden et al. 2005) and *in vitro* test systems (Teeguarden and Barton 2004).

A separate set of challenges has prevented widespread alignment of particle and nanoparticle exposures between *in vitro* and *in vivo* systems. The deposition of particles in the upper and lower airways of rodents and nonhuman primate toxicity-testing systems and of humans is governed by physical processes (gravity, diffusion, and impaction), breathing patterns, airway structure (size, branching pattern, and geometry), and particle characteristics (size, shape, and density). Similar processes affect gravitational and diffusional transport and eventual particle deposition on target cells in liquid cell-culture systems and include agglomeration capacity; particle size, shape, density, and agglomeration size and density; media height; and diffusion (Teeguarden et al. 2007; Hinderliter et al. 2010; Cohen et al. 2014; DeLoid et al. 2014). Until recently, toxicity data on particles from *in vivo* and *in vitro* systems were compared on different exposure scales—for example, air concentrations and liquid cell concentrations (Sayes et al. 2007)—and this potentially obscured relationships between biological effects in the systems. More recently, direct measurement of target-cell doses has become more common. In addition, with the advent of computational tools that can capture the unique kinetics of particles in solution (Hinderliter et al. 2010) and of supportive experimental methods (Davis et al. 2011; Cohen et al. 2014), computational estimation of cellular doses in *in vitro* systems is becoming more common. With similar tools for measuring or calculating lung-tissue doses of particles after inhalation exposure (Anjilvel and Asgharian 1995; Asgharian and Anjilvel 1998; Asgharian et al. 1999, 2001, 2006, 2012; Asgharian 2004; Asgharian and Price 2007), approaches that allow comparison of *in vitro* and *in vivo* models of experimental particle toxicity have emerged (Teeguarden et al. 2014). The consistency of observed effects between the *in vitro* and *in vivo* systems might be revealed by making comparisons with a consistent, biologically relevant measure of exposure. For example, iron oxide nanoparticles were shown to cause expression of the same cytokines in macrophages *in vitro* and in mouse lungs *in vivo* when exposures were compared on a particle mass or cell basis.

Research in and development of new methods and more frequent application of existing methods to produce consistent measures of biologically appropriate exposure for toxicity across various test and receptor systems is a potentially high-value application for exposure science.

Improving Exposure Assessment for Epidemiological Studies

Causal inference based on epidemiological evidence can be strengthened when information on health outcomes is combined with clear measures of exposure at the biological site of action or a surrogate for the site of action (such as serum) that is temporally related to the causative biological events. Although that assertion is based on fundamental principles of pharmacology, it is not true that internal exposures are univer-

sally better than external exposure for purposes of assessing associations or inferring causation. External-exposure measures have been and will continue to be sufficient, and in some cases superior to internal-exposure measures, for example, where portal-of-entry effects are involved or large population-scale exposure assessments are necessary and internal-exposure assessments are impractical. Reducing or eliminating exposure misclassification and broadening exposure assessment to identify new chemicals that might be causative agents or confounders of existing associations would substantially strengthen the interpretation of epidemiological studies and improve their value for public-health decision-making.

Several advances in the field of exposure science are particularly well suited for improving exposure assessment for epidemiological studies. High-throughput targeted and nontargeted analytical-chemistry tools and new matrices for exposure assessment (such as hair, teeth, and nails) are together expected to offer more temporally relevant exposure assessment of many more chemicals and expand exposure assessment over the full life span. Emerging high-throughput computational-exposure models of external exposure will provide exposure estimates that complement those made through expanded biomonitoring programs. Personal biomonitoring and sensor wristbands (O'Connell et al. 2014a,b) offer an unparalleled opportunity to characterize individual exposures and provide temporally and spatially resolved data for understanding patterns of exposure, variability, and the role of behavior and activity levels on exposure. PBPK models could improve exposure assessment by

- Reconstructing exposures from limited biomonitoring samples on the basis of pharmacokinetic understanding (Tan et al. 2006, 2012; Yang et al. 2012).
- Translating external exposures or biomonitoring data into more biologically relevant internal exposures (Teeguarden et al. 2013).
- Reducing the likelihood of reverse causation in epidemiological studies by more clearly delineating the sequences of chemical-induced physiological changes that lead to disease states (Verner et al. 2015; Wu et al. 2015)
- Accounting for population variability that is characterized directly or through the application of pharmacogenomics approaches (Teeguarden et al. 2008; EPA 2010; Ginsberg et al. 2010).

The greater availability of internal-exposure information obtained from direct biomonitoring of human populations or from a combination of computational tools would be of particular value by providing human exposure concentrations at the site of action (tissue or blood). Such information could be compared with measurements in animal and cell-culture studies and might enhance causal inferences derived from epidemiological studies.

Exposure-Based Screening and Priority-Setting

Several exposure-based priority-setting approaches that benefit from the emerging exposure-science tools and data streams have been developed. In an exposure-based approach, chemicals in the top exposure category are assigned a higher priority for additional tiered toxicological, hazard, or risk assessment than those in the low exposure category; this provides a reproducible, transparent, and knowledge-based framework to inform decisions for testing priorities (Egeghy et al. 2011; Wambaugh et al. 2013, 2014). The European Food Safety Authority and WHO have reviewed the threshold-of-toxicological-concern (TTC) approach as a screening and priority-setting tool that can be used for chemical assessments in cases where hazard data are insufficient and human exposure can be estimated (EFSA 2016). The TTC approach is used principally as a screening tool to assess low-dose chemical exposures and to identify those on which further data are necessary for assessing human health risk.⁷ In some cases following certain re-

⁷The committee notes that TTC approach depends on the set of chemicals used to establish the toxicity distribution that is used to derive the TTC value. The ability of the TTC approach to screen chemicals properly will depend

quirements, “exposure-based waiving” for toxicity testing or “exposure-based adaptation of information requirements” approaches can be considered under the European Registration, Evaluation, Authorisation and Restriction of Chemicals legislation (Vermeire et al. 2010; Rowbotham and Gibson 2011). Exposure-based waiving has also been used to propose acceptable exposure levels determined on the basis of generalized chemical-toxicity data and without chemical-specific toxicity data. Such approaches might be useful in making initial decisions about the public-health importance of chemical exposures in lieu of complete exposure and hazard data. Within the bounds of uncertainty and variability of the data, some immediate decisions could be made about the low potential for risk posed by exposures below preselected “critical levels” (Vermeire et al. 2010; Rowbotham and Gibson 2011). Cumulative exposures to chemicals in specific classes might move some chemicals up in priority—an outcome of improved exposure data. Structure-based alerts and TTCs can be applied in such screening contexts to complement the exposure-based decision-making process. EPA recently demonstrated integration of nontargeted and targeted chemical analysis of house-dust samples for exposure-based and bioactivity-based ranking of chemicals for further biomonitoring or toxicity testing as shown in Figure 2-7 (Rager et al. 2016).

Biomonitoring data and environmental-monitoring data on most chemicals in commerce are missing or insufficient for exposure-based decision-making. Application of advanced biomonitoring, personal monitoring, and computational exposure-science tools described in this chapter can support high-throughput screening-level exposure assessment and exposure-based priority-setting for later toxicity testing. Exposure models can be applied to screen large numbers of chemicals in commerce and set priorities among specific chemicals or chemical classes on which there are no or few toxicity-testing data (McLachlan et al. 2014). Chemicals that have predicted high concentrations in humans and environmental media can then be used to identify toxicity-data gaps and set priorities for toxicity-testing for risk-based applications. The committee notes that priority-setting based only on exposure might assign a lower priority to chemicals that might be given a higher priority on the basis of toxicity or risk.

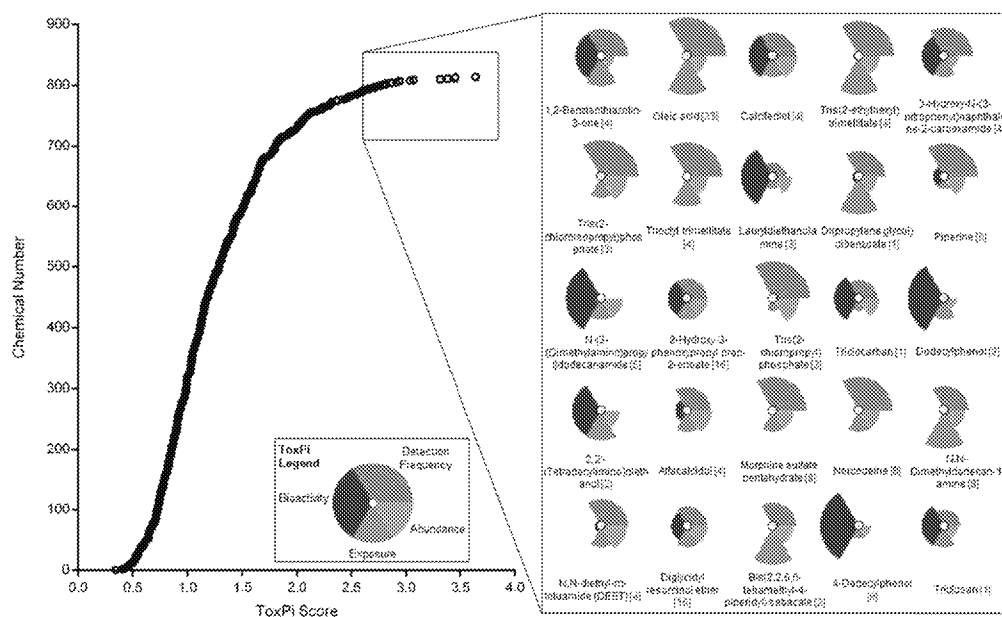


FIGURE 2-7 Data from nontargeted and targeted analysis of dust samples were used with toxicity data to rank chemicals for further analysis and testing. Source: Rager et al. 2016. Reprinted with permission; copyright 2016, *Environment International*.

on whether the toxicities of the chemicals of interest are well represented by the toxicities of the chemicals used to establish the distribution.

Translation of high-throughput data into risk-based rankings is an important application of exposure data for chemical priority-setting. Recent advances in high-throughput toxicity assessment, notably the ToxCast and Tox21 programs (see Chapter 1), and in high-throughput computational exposure assessment (Wambaugh et al. 2013, 2014) have enabled first-tier risk-based rankings of chemicals on the basis of margins of exposure—the ratio of exposures that cause effects (or bioactivity) to measured or estimated human exposures (Wambaugh et al. 2013, 2014; Wetmore et al. 2013, 2014; Shin et al. 2015). Building on work by Wetmore et al. (2012) and Rotroff et al. (2010), Shin et al. (2015) demonstrated a high-throughput method for screening and setting priorities among chemicals on the basis of quantitative comparisons of exposure data with in vitro bioactivity data (bioactivity quotients); this is similar to the margin-of-exposure approach used in risk priority-setting. They used human intake rates estimated with computational exposure models and toxicokinetic models for the in vitro–in vivo extrapolation of ToxCast toxicity data and identified 38 of 180 chemicals for which total estimated exposures equaled or exceeded the estimated oral dose expected to result in blood concentrations that cause a 50% response in an in vitro toxicity-testing system. Population variability due to differences in metabolic capacity was incorporated into the process (Wetmore et al. 2014). Screening-level exposure assessment was used to establish margins of exposure for that group of chemicals for purposes of priority-setting. The committee notes, however, that limitations of such analyses (see section “New Approaches for Assessing Biochemical and Physiological Determinants of Internal Exposure” above) need to be taken into account. Although exposure estimates that exceed in vitro effect estimates might not be conclusive evidence of risk and exposures that fall below in vitro activities might not be conclusive evidence of no risk, the committee sees the potential for the application of computational exposure science to be highly valuable and credible for comparison and priority-setting among chemicals in a risk-based context.

Human-exposure data on a much larger suite of chemicals than is now available would provide important new data for guiding selection of chemicals and exposure concentrations for hazard testing and mechanistic toxicology. The rapid expansion and use of high-throughput in vitro methods for hazard assessment and mechanistic studies presents a growing opportunity to test chemicals for bioactivity at human-exposure levels—levels lower than those typically used in traditional toxicity-testing studies. In vitro test systems—which are less subject to statistical-power limitations, are less expensive, and have fewer ethical considerations than whole-animal studies—might be better suited for testing exposures lower than those in traditional animal studies. Recent animal studies, however, provide useful examples of applying human exposure information to in vivo test systems. For example, recent studies have included exposures at or near those experienced by humans in animal-testing protocols for genistein and synthetic estrogens (NTP 2008; Delclos et al. 2009, 2014; Rebuli et al. 2014; Hicks et al. 2016). For those animal studies, exposures were selected on the basis of measured serum concentrations obtained in pilot animal studies, values estimated with pharmacokinetic models, and measured or estimated serum concentrations in humans. The use of target-tissue exposures or biologically relevant accessible proxies, such as serum, for selecting can in some cases be of greater relevance than the use of external exposure measures. Thus, there is an opportunity to apply many of the new tools described in this chapter—expanded biomonitoring, new biological matrices, and high-throughput computational exposure models—as a guide for the selection of exposures for use in toxicity testing (Gilbert et al. 2015).

Identifying New Chemical Exposures for Toxicity Testing

The totality of exposure that makes up the exposome includes registered chemicals that are used in commerce, their environmental and metabolic degradation products, and endogenously produced chemicals. Traditionally, hazard-testing paradigms focus on satisfying regulatory needs for supporting product registration and contain guidelines for testing commercial chemicals, not their degradation products, metabolites, or similar chemicals produced endogenously. Identification of chemicals that make up the latter groups of untested chemicals has become a key goal of federally funded exposure-science programs, such

as the Children's Health Exposure Analysis Resource. Owing to advances in high-throughput nontargeted analysis (Fiehn 2002; Park et al. 2012; Go et al. 2015; Mastrangelo et al. 2015; Sud et al. 2016), exposure science is in a more effective position for discovery-based exposure assessment. Combined with environmental-degradation studies to identify novel chemicals, higher-throughput targeted analytical methods also contribute to overall exposure discovery for toxicity testing. For example, researchers in the Oregon State University Superfund Research Program recently discovered novel oxygenated and nitrogenated polycyclic aromatic hydrocarbons produced by conventional remediation methods and have subjected these environmental degradation products to toxicity testing (Knecht et al. 2013; Chibwe et al. 2015; Motorykin et al. 2015). In collaboration with academic scientists, EPA (Rager et al. 2016) recently demonstrated a workflow for nontargeted analysis of house dust with a transition to targeted analysis (measurement of specific target analytes) for ToxCast chemicals and use of frequency of detection information on chemicals as exposure data for priority-setting shown in Figure 2-8 below. The committee sees the use of nontargeted and targeted analysis as one innovative approach for identifying and setting priorities among chemicals for additional exposure assessment, hazard testing, and risk assessment that complements the current hazard-oriented paradigm.

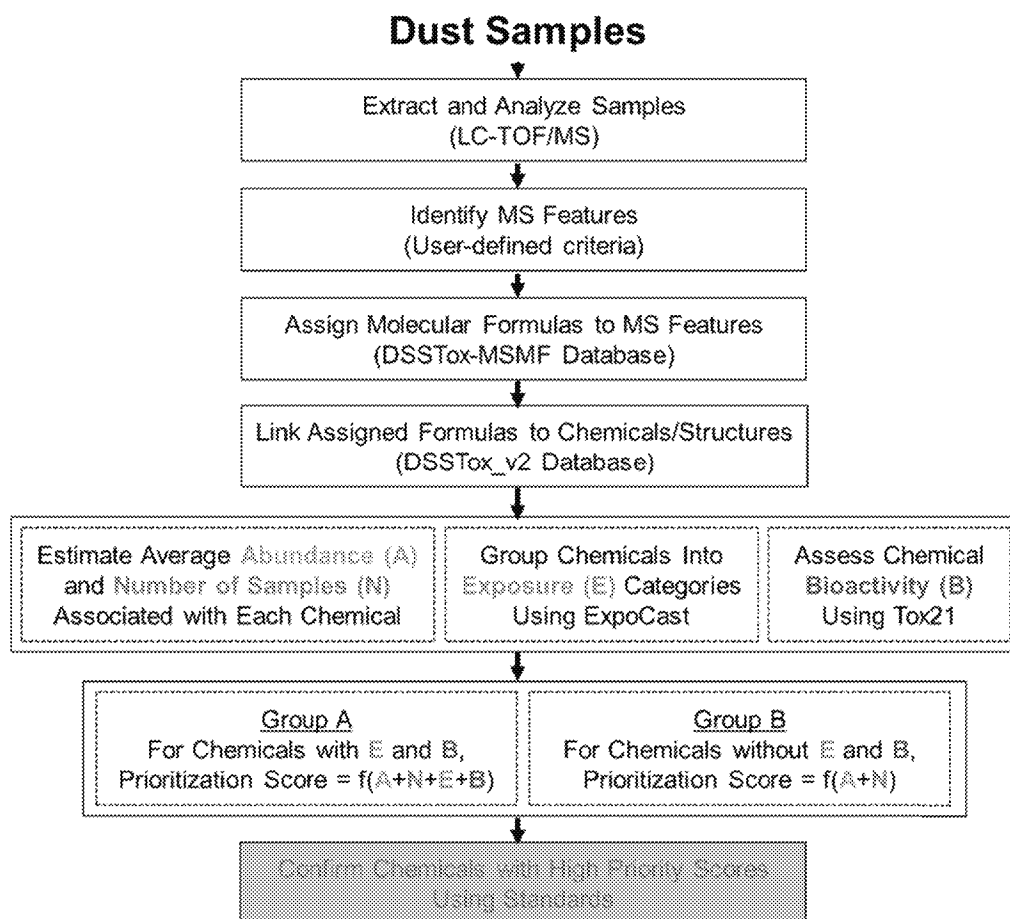


FIGURE 2-8 Workflow for nontargeted and targeted analysis of the house-dust exposome for chemical priority-setting and testing. Abbreviations: DSSTox-MSMF, Distributed Structure-Searchable Toxicity Database-Mass Spectroscopy Molecular Formula; LC-TOF/MS, Liquid chromatography time-of-flight mass spectroscopy; and MS, mass spectrometry. Source: Rager et al. 2016. Reprinted with permission; copyright 2016, *Environment International*.

Predicting Exposure to Support Registration and Use of New Chemicals

About 1,000–2,000 chemicals are introduced into commerce each year (EPA 2004). For newly introduced chemicals, exposure assessment means forecasting likely environmental concentrations or total daily human exposures resulting from expected uses and is not a regular part of the decision-making process. The case of methyl tertiary-butyl ether, a gas additive introduced without fate and transport calculations and later found to be widely distributed in the environment, is a poignant example of the value of predictive exposure modeling (Davis and Farland 2001). A recent NRC report, *A Framework to Guide Selection of Chemical Alternatives*, found that despite the known importance of exposure, many frameworks for selecting chemical alternatives downplay its importance and focus on inherent hazards posed by chemicals (NRC 2014). The committee that prepared the report recommended an increased emphasis on comparative exposure assessment and stated that inherent hazard should be the focus only in cases where the exposure routes and concentrations of the chemical of concern and its alternatives are not expected to differ substantially; that is, equivalent exposures should not be automatically assumed. And, it recommended greater reliance on physicochemical data and modeling tools, when high-quality analytical data on exposure are unavailable, to aid in predicting the partitioning of contaminants in the environment and the potential for their persistence, bioaccumulation, and toxicity. Although approaches that are based on both hazard and exposure data are preferred, approaches that are based principally on exposure or hazard data will continue to be valuable depending on the decision context.

Tools to predict chemical properties (environmental or tissue-partitioning properties), stability (degradation and metabolism half-lives), and proposed use scenarios can be used to set parameter values for exposure models that are used to predict concentrations in environmental media and humans, over life spans, and on local and national scales. The estimated concentrations can guide selection of toxicity-testing exposures and can be compared with emerging toxicity data for risk-based assessments. Green-chemistry modeling initiatives can be applied to prescreen candidate chemicals according to the likelihood of biodegradation (Boethling 2011). Candidate chemicals can also be screened by applying more comprehensive methods that consider environmental fate and transport and various chemical use scenarios (release pattern and quantities) (see, for example, Gama et al. 2012). Confidence in the prescreening methods will be greatest when the models and tools cover the applicability domain of the chemicals that are being evaluated and when the tools have already been shown to be effective in predicting fate and transport of chemicals that have similar properties (for example, structural similarity or similar use categories). Hence there is a need to test and evaluate exposure modeling tools and data streams systematically with existing commercial chemicals to foster confidence in applying the same and emerging tools for new premarket chemicals.

Identifying, Evaluating, and Mitigating Sources of Exposure

For chemicals that have multiple relevant exposure pathways, it can be challenging to identify and rank exposure sources for mitigation. Exposure models can be used to reconstruct and identify the sources, behaviors, and pathways that are driving exposures to a particular stressor. Good examples of emerging computational exposure tools that can be used to trace exposures to sources are exposure models for consumer products (Gosens et al. 2014; Delmaar et al. 2015; Dudzina et al. 2015) and exposure models and frameworks that combine far-field and near-field pathways for aggregate human exposure assessments (Isaacs et al. 2014; Shin et al. 2015). For example, Shin et al. (2014) combined exposure models and human-biomonitoring data for nine chemicals to estimate the proportions of total production volumes that are used in selected use categories that correspond to exposure pathways. The models can be used to develop targeted strategies to reduce or virtually eliminate exposures to a particular stressor. For some chemicals, such as those used in pharmaceuticals and personal-care products, the dominant exposure pathways and chemical use rates are relatively obvious, and source mitigation, if necessary, might be relatively straightforward.

The combination of sensor technologies, including personal sensors, with GIS data systems offers new capabilities to identify sources of exposure. Personal sensors—for example, cell-phone-based sulfur oxide and nitrogen oxide sensors—use native GIS systems to collect real-time exposure data, which can be used to identify locations with high exposures and the source locations that contribute to the exposures. Remote sensing can identify high-exposure locations and source locations on a regional or population scale by mapping pollutant concentrations and identifying exposure patterns that might be related to sources.

Some chemicals and materials are poorly degraded and persist in the environment long after production and use are stopped. Some of the highly persistent chemicals also have long residence times in the human body. It can take years or decades for exposures to decline substantially after regulatory action is initiated. Accordingly, highly persistent chemicals that show unacceptable risk should have high priority for mitigation. Models and supporting experimental studies that screen for rates of chemical degradation in environmental media and overall persistence in the environment and in humans can be used to identify persistent chemicals before commercial use and prevent or mitigate potential exposure by finding alternatives.

Emerging exposure-assessment tools can also be used to mitigate sources of exposure to chemicals that cannot be identified confidently. Specifically, nontargeted analysis of environmental samples—air, dust, water, and soil—can be combined with analysis of ecological or human biomonitoring samples to select analytical features that represent internal exposures of potential concern. Geographical mapping of relative concentrations or detection frequency in environmental and human samples can lead to source identification that might in turn help to identify the chemical classes.

Assessing Cumulative Exposure and Exposure to Mixtures

Humans, animals, plants, and other organisms are exposed to numerous stressors that vary in composition and concentration over space and time. For the most part, traditional toxicity testing has been conducted largely on single chemicals, so there are important uncertainties in assessing potential short-term and long-term effects of exposures to a mixture. That issue is a well-recognized concern for chemical assessment. With advances in exposure data streams and the potential for high-throughput toxicity screening, there are opportunities to address the uncertainty related to potential effects of mixture exposures better. Measurements obtained from human tissue and from environmental media to which humans are exposed can be used directly or indirectly to formulate environmentally relevant concentrations of mixtures for toxicity screening and testing. For example, internal concentrations of persistent organic pollutants from *in vivo* exposure of humans (silicone implants) were used to determine and test mixture toxicity in *in vitro* assays (Gilbert et al. 2015). It is also possible to use environmental-monitoring data (sampled water concentrations) to formulate exposure mixtures for toxicity testing (Allan et al. 2012), including approaches that consider population variability in responses to environmentally relevant chemical-mixture concentrations (Abdo et al. 2015). The substantial advances in analytical chemistry noted in this report are producing more complete data on the extent of cumulative exposure to chemicals. Personal sampling devices, such as wristbands and air-sampling devices, provide data on complex cumulative exposures of individuals. -Omics tools appropriate for measuring the aggregate biological response to cumulative exposures to chemical classes that act through similar mechanisms can be combined with measures of real-world cumulative exposures to assess the effects of cumulative exposures more comprehensively. Aggregate-exposure model calculations for individual chemicals could be combined to obtain estimates of cumulative exposures to mixtures, for example, by using models of exposure to consumer products that are supported by databases of chemical concentrations in the product and product-use rates. The exposure-model calculations could be used to address mixture exposures and potential toxicity; this approach would require mixture-toxicity data or mixture-toxicity models for risk-based assessment. For that case, estimating exposure to a mixture of chemical stressors for risk-based assessments is theoretically possible. The reliability of and confidence in the exposure calculations require further evaluation, and methods for including metabolites and nonchemical stressors in cumulative risk-based evaluations are also required.